PREVENTION OF BOVINE MASTITIS THROUGH A COMBINATION OF COW COMFORT AND MASTITIS INTERVENTIONS ON SMALLHOLDER DAIRY FARMS IN KENYA

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Examiners

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Abstract

Smallholder dairy farming is a critical sector supporting many households' income in rural Kenya. The farmers are faced with mastitis and cow comfort challenges that impair the optimal performance of their dairy cattle. A cross-sectional study was conducted on 118 cows in their first two months in milk on 109 SDF in Kenya to investigate the relationships among various cow and farm management parameters and subclinical mastitis (SCM) specific to smallholder dairy farms (SDF). The stall floor comfort level and various mastitis prevention measures were assessed. Individual quarter SCM was assessed using the California Mastitis Test (CMT). Univariable and multivariable logistic regression models were fit to determine management factors associated with cow-level SCM.

Farm-level, cow-level and quarter-level prevalence of SCM was 45.9% (50/109), 43.2% (51/118) and 21.9 % (103/471), respectively. The proportion of stalls scored as dirty was 33.1% while 49.1% of cows had dirty legs. Only 10.1% of farms were using either disinfectant teat dip or dry cow therapy (or both) to prevent mastitis. Low parity and poor stall hygiene were significantly associated with occurrence of SCM. At high daily milk yield (>10 Kg/day), the probability of having SCM was higher in cows housed in a shed with a dirty versus clean alleyway, with no significant difference at low daily milk yield.

A randomized controlled trial was conducted to assess compliance with cow comfort recommendations and their impact on cow comfort in Meru Kenya. A total of 114 farms with 124 cows were recruited. The status of each sleeping stall used by each respective cow included in the study was evaluated on the first farm visit. Farms were randomly placed into either intervention (n=74) or control groups (n=46), and intervention farms were given farm-specific cow comfort recommendations. Farms were revisited after 2 months and compliance to the

recommendations given were assessed and given a percentage compliance score. A discomfort index was arithmetically calculated from stall hygiene and stall hardness. A mixed logistic regression model was utilized to determine associations between compliance and comfort recommendations. Multivariable linear regression models were used to determine specific associations with the discomfort index.

In the 114 cows on the110 SDFs that remained in the trial, the overall compliance to the recommendations was 49.0%. Improving stall hygiene (83.3%), creating ample leg space (76.9%) and making the stall soft and dry (76.3%) accounted for recommendations with the highest item compliance scores. Bedding type, neck rail positioning, and the interaction between intervention group and visit number (p=0.01) were significantly associated with discomfort index. In the final mixed logistic regression, type of recommendation and number of recommendations given were significantly associated with compliance of individual recommendations.

A second randomized controlled trial was conducted on the same 110 SDFs with 114 cows to determine compliance with both milking hygiene and cow comfort recommendations (the intervention), factors associated with compliance, and the impact of both milking hygiene and cow comfort recommendations on occurrence of subclinical mastitis in SDFs. Farmers were randomly placed into either intervention (n=74) or control groups (n=46). SCM was evaluated using CMT, and intervention farms received farm-specific milking hygiene recommedations on the first farm visit. Compliance was again assessed 2 months later and SCM was reassessed with CMT.

The mean compliance for milking hygiene recommendations was 77%, while average compliance for both milking hygiene and cow comfort recmmendatons was 63.2%. The type of

recommendation given and number of recommendations given were significantly associated with compliance with the recommendations. The principal farmer and their age group were significantly associated with quarter CMT scores improving between the first and second visits.

In conclusion, giving fewer recommendations that were easier to implement was significantly associated with higher compliance to recommendations. Women and older farmers were more likely to implement recommendations. Improving cow comfort (especially bedding type and neck rail positioning) and milking hygiene protocols (especially iodine teat dip use and washing hands before and between cow milking) through farmer-implemented changes in response to recommendations can lead to a significant increase in cow comfort and a significant decrease in subclinical mastitis.

Dedication

I dedicate this work to all smallholder dairy farmers in Kenya to be a guide towards improving mastitis control, cow comfort and sustainable productivity from their cows.

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I am grateful to God for presenting me the chance and taking me through the journey of this work.

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Chapter 1: Introduction

1.1 Overview

Mastitis is the most economically challenging production disease affecting dairy cattle around the world (Abebe et al., 2016; Awale et al., 2012a; Bardhan, 2013). Mastitis is the inflammation of the parenchyma of mammary tissue and is characterized by physical, chemical and usually bacteriological changes (Radostits et al., 2007). It is associated with losses from reduced milk production, deterioration of milk quality and increased costs in managing the disease (Hogeveen et al., 2011; Karimuribo et al., 2006; Mungube et al., 2005; Petrovski et al., 2006). The occurrence of the disease results from an interplay between the infectious agents, the host's immune system, and environmental factors (Sandeep & Anirban, 2011).

Based on severity of signs observed, mastitis can be classified into clinical and subclinical mastitis (Radostits et al., 2007; Smith et al., 1985). Clinical mastitis is characterized by cardinal signs of inflammation in the udder, and abrupt reduction in milk production, with abnormal visible changes in the milk, making the milk unsuitable for sale or consumption. In subclinical mastitis (SCM), there are no detectable changes in the milk and udder. Therefore, it is not easy to detect subclinical mastitis without testing the milk. However, there is an increase in the somatic cell count in subclinically infected milk which can be detected by cell counters and the California Mastitis Test (CMT) (Dingwell et al., 2003). Subclinical mastitis is also an expensive production disease in dairy farming, particularly because it poses the challenge of early detection, and thus can remain in a herd long-term, causing chronic declined milk production and exposing other cows to the risk of infection (Mungube et al., 2005).

Bacteria are the primary etiological agents of bovine mastitis (Bradley, 2002). Based on the source of the infection, bacterial mastitis is also traditionally categorized into environmental and contagious mastitis (Cervinkova et al., 2013). Environmental mastitis is caused by bacteria whose primary reservoir is a unhygienic housing and/or milking environment (Klaas & Zadoks, 2018). Most cases of mastitis from environmental pathogens are of clinical nature and of short duration (Harmon, 1994). Commonly isolated bacteria include *Streptococcus uberis, Streptococcus dysgalactiae* and members of Enterobactericiae family, such as *Eschericia coli*.Conversely, contagious mastitis is caused by bacteria whose primary reservoir is an infected udder, and the bacteria are transmitted between quarters within and between cows during the milking process (Radostits et al., 2007). These include Staphylococcus species and Streptococcus *agalactiae*. Contagious mastitis bacteria are frequently associated with chronic subclinical mastitis (Sharma et al., 2011).

The first line of treatment of bacterial mastitis is stripping the infected quarter to remove the bacteria, which can be augmented by use of antimicrobials, where indicated, either systemically or as an intra- mammary (IMM) infusion into the teats. Antimicrobials have been used in treating mastitis for more than 50 years (Ruegg, 2017). In recent years, their use has had varying degrees of effectiveness due to insufficient understanding of the pathogens involved, inadequate treatment regimens (suboptimal route, amount, frequency and/or duration), and antimicrobial resistance, among other factors (Jasovský et al., 2016a; Ruegg, 2017).

Antimicrobial resistance (AMR) has emerged as a global threat to the effective use of antimicrobials in the treatment of mastitis, and the current global burden of disease is higher than the 700,000 human deaths per year estimated by WHO and UN (WHO., 2019). AMR occurs where bacteria develop mechanisms to evade the action of antimicrobials used in treatment of mastitis (Ventola, 2015). This challenge has spurred global research interests toward reduction of antimicrobial use and finding more sustainable and effective methods of controlling bacterial infections including mastitis in dairy farms.

Management factors have been explored in the control of mastitis in dairy cows in high-income and middle- to low-income countries (Halasa et al., 2007; Hogeveen et al., 2011; Mungube et al., 2005). Improving the hygiene of the environment, and in the milking process, is known to reduce occurrence of environmental and contagious mastitis, respectively (Awale et al., 2012a). Studies have also demonstrated that poor cow comfort is associated with low cow immunity as well as predisposing cows to injuries (Bernardi et al., 2009; Keyserlingk et al., 2009; Lombard et al., 2010a). However, the role of cow comfort in the control of mastitis has not been explored or studied in detail in low-income countries such as Kenya. A previous study showed that lying down time as a measure of comfort was significantly improved from implementing a set of cow comfort recommendations in Kenya (Kathambi, et al., 2019) but the study did not focus on the impact of comfort recommendations on mastitis. This thesis is aimed at investigating compliance with mastitis control and comfort recommendations in smallholder dairy farms (SDFs) in Kenya and the impact of compliance in occurrence of subclinical mastitis.

1.2 Literature review

This section gives an outline of research that has been done within the topics covered in this thesis. The focus is mainly on research done on SDFs in low-income countries covering mastitis control and cow comfort, but will also highlight relevant work done in high-income countries. These broad topic areas are broken down into sub-headings for a review of the topics of interest, with more focus drawn towards subclinical mastitis since it is of primary interest to the thesis.

1.2.1 Smallholder dairy farms in low-income countries

Smallholder dairy farms (SDFs) in Kenya are characterized by a number of features which are typical of such farms in many low-income countries. Commonly, less than 5 milking cows are reared in a simple housing structure usually made from timber or poles and less often with a wall made of bricks/ blocks (Gitau et al., 1994). The dairy business is often the sole source of income for the household. The family members provide labor for all operations on the farm. Milk is marketed mostly raw through dairy cooperatives and the proceeds provide a source of livelihood for the family.

In low-income countries, SDFs make up a majority of the dairy farms and account for most of the milk produced. In Kenya, it is estimated that around 80% of the milk produced comes from SDFs, accounting for 56% of dairy cows kept. These SDFs are contributing approximately 6% of the national gross domestic product, with an estimated annual growth rate of 4.1% per year in production (Bonilla et al., 2017; USAID & GoK, 2009).

It is not uncommon to find cows in SDFs kept in suboptimal housing conditions (Aleri et al., 2012; Kawonga et al., 2012a; Nkya et al., 2007) or undergoing inadequate mastitis control protocols (Gitau et al., 1994), leading to substantial mastitis problems. Among other factors, these mastitis problems are a function of inadequate resources for required mastitis prevention protocols, as well as low knowledge levels among the SDF farmers (Aleri et al., 2012; Kawonga et al., 2012b; Nkya et al., 2007).

1.2.2 Mastitis etiology and its occurrence on SDF in developing countries

Mastitis is prevalent among SDFs in low-income countries, and in Kenya, Mbindyo et al., (2020) revealed a cow-level prevalence of 80% from CMT. Mureithi and Njuguna (2016) found a quarter-level prevalence of 56% and a corresponding 70% cow level prevalence in one part of Kenya, while another study (Gitau et al., 2014) found 52% and 61% cow-level prevalences on CMT from 2 different districts in Kenya. This evidence indicates the importance of mastitis in SDFs in Kenya and calls for more attention in efforts towards its control.

Previous studies have sought to identify pathogens involved in mastitis etiology on SDFs. In a study conducted in Colombian SDFs, *Streptococcus agalactiae* was the predominant organism cultured (47%), followed by coagulase-negative *Staphylococcus* species (14.3%) and *Staphylococcus aureus* (13%) (Ramírez V. et al., 2001a). A study conducted in the same area 7 years later established *Staphylococcus aureus* as the chief organism isolated (Calderón & Rodríguez, 2008).

Several studies conducted in rural SDFs in India found *S. aureus* as the chief etiological agent of cow and buffalo mastitis (Khan & Muhammad, 2005; Sharma et al., 2011). Similar findings have been established in Kenya (Gitau et al., 2014; Mureithi & Njuguna, 2016; Odongo & Ambani, 1989; Shitandi & Sternesjö, 2004) where *S. aureus* was found to be the predominant etiological agent. A recent study in central Kenya (Mbindyo et al., 2020) found coagulase-negative *Staphylococcus* species to be predominant, with *S. aureus* coming third after *Streptococcus* species.

With the high prevalence of mastitis, and predominance of *S. aureus*, SDFs in Kenya and other low-income countries are faced by a serious threat to improved milk productivity. There is need to find and utilize cost-effective and sustainable mastitis control strategies in order to address the challenge. Such strategies should be tried and verified to be effective in order to encourage their widespread adoption.

1.2.3 Losses attributed to occurrence of mastitis on SDF

Mastitis is a disease of economic concern to dairy producers globally (Sharifi et al., 2015). In Europe, costs have been estimated at between 61 and 97 Euros per cow per lactation, with large variations across farms (Hogeveen et al., 2011). Losses are incurred in the form of reduction in milk production, veterinary costs in treatment of the disease, and rejection of milk due to milk quality issues, as well as premature culling of cows with intractable mastitis problems (Sharifi et al., 2015). In a study in Iran, the economic losses of mastitis were 492 USD per cow per lactation, comprising of decline in milk production (62.2%), milk loss within 7 days of infection (24.1%) and cost of treatment (6.8%). In Canada, an estimated loss of 662 CAD per cow per year has been recorded (Aghamohammadi et al., 2018). In Colombia, losses due to subclinical mastitis in small and medium dairies have been estimated at 800 USD per cow per lactation (Romero et al., 2018). Unfortunately, mastitis often occurs in high-producing cows, and when it occurs, SDFs are prone to lose a significant source of their livelihoods.

Subclinical mastitis accounts for an estimated 15-40 times more losses compared to clinical mastitis (Jones et al., 1998; Mdegela et al., 2009). *Staphylococcus aureus* is the most frequently isolated etiological agent for subclinical mastitis (Islam, et al., 2012; Jones et al., 1998; Olsen et al., 2006; Shitandi & Sternesjö, 2004). Studies have shown that *S. aureus* is able to form micro-abscesses in the mammary tissue which help it evade the cows immune response as well as antibiotics used in treatment (Abdi et al., 2018; Jones et al., 1998). As a result, the organism is shed intermittently in milk, and an infected cow becomes a source of infection to others in the herd (Sears et al., 1990).

Since cows are a source of milk for human consumption, a close link is created between cow health and human health. A number of mastitis pathogens have been found to have zoonotic potential (Fernandes et al., 2011; Schans et al., 2009). It is necessary for farmers and animal health professionals to take this zoonotic concern into account when planning and implementing farm mastitis control protocols. Additionally, antimicrobial medicines used in mastitis treatment, among other infections, can have a long residual effect, and if withdrawal periods are not adhered to, they could find their way into the human food chain, creating a link for AMR between animals and humans. In these ways, mastitis poses a risk to human health, and its control should be a matter of priority not only to farmers but also to governments.

1.2.4 California Mastitis Test and culture for mastitis diagnosis

It is important to detect the presence of mastitis in a herd as soon as it occurs in order to prevent its spread to multiple cows. Considering that subclinical mastitis cannot be diagnosed from physical changes in the udder, it may take a while to be identified. Even after a diagnosis, treatment attempts are sometimes futile when the specific organism has not been identified. CMT is an inexpensive yet reliable cow-side test which is relatively easy to run and interpret results. It can be conducted for individual quarter milk, composite cow milk, or bulk milk. On the farm, it is conducted on individual quarters, and treatment is directed at positive quarters only. However, interpretation of results is somewhat subjective, which can yield discrepancies between evaluators (Moroni et al., 2018a).

CMT is based upon presence of somatic cells in the milk, and the number of these cells, denoted as somatic cell count (SCC), is an indicator of inflammation in the mammary parenchyma (Dingwell et al., 2003; Sharma et al., 2011). An elevated SCC in milk has a negative impact on milk quality and quantity from a quarter (Hagnestam-Nielsen et al., 2009). SCC, reported in number of cells per milliliter (cells/ml) of milk, is used as a diagnostic figure for SCM (Smith & Hogan, 1999). Past studies have identified a cutoff of 200,000 cells per liter as indicative of mastitis (Hagnestam-Nielsen et al., 2009; Hillerton, J.E., 1999; Smith & Hogan, 1999).

Identifying the organism involved is critical for informing the treatment choice and to guide prognosis. Culture and identification should be done for this purpose. Milk samples collected aseptically should be obtained to ensure the results are accurate and reliable. Samples can be fresh, chilled or frozen, depending on the duration from collection to delivery to the lab for culture. Fresh samples are usually preferable for better odds of isolating bacteria. Some bacteria have been known to be prone to freeze-thaw activity, which is possible in frozen samples. A previous study (Schukken et al., 1989) found freezing and length of storage resulted in a decrease in number of samples that had growth of *Escherichia coli* and *Actinomyces pyogenes* (currently called *Trueperella pyogenes*). The same study found that there was an increase in number of samples that had growth of coagulase negative staphylococci (CNS) after freeze-thaw cycles, and no effect on *S. aureus* (Schukken et al., 1989). Some bacteria have been considered common laboratory work space contaminants, including *Staphylococcus epidermidis* and *Bacillus subtilis* (Ghayoor et al., 2015).

1.2.5 Factors associated with occurrence of subclinical mastitis on SDF

Mastitis occurrence involves the interplay between the pathological agents, cow factors, and the environment. Bacterial characteristics, such as virulence and ability to colonize mammary tissue, affect the occurrence and severity of mastitis, and these characteristics can vary from farm-to-farm, depending on the history of contamination of a farm from other farms (Abdi et al., 2018; Sawant et al., 2009).

Cow factors, such as breed, parity, milk yield and stage of lactation, have also been linked to higher prevalence of mastitis on SDFs (Dego & Tareke, 2003; Lakew et al., 2009; Mureithi & Njuguna, 2016). Older animals are more likely to get mastitis compared to younger ones, while high-producing cows are at higher risk compared to those producing less milk. Heredity has also been incriminated as a predisposing factor for mastitis (Parker Gaddis et al., 2014).

In the SDF environment, poor hygiene in the cow's stall and in the milking process have been associated with increased mastitis prevalence (Awale et al., 2012a; Dego & Tareke, 2003; Lakew et al., 2009). Contagious mastitis bacteria have been transmitted from cow-to-cow when milking equipment or milkers' hands are contaminated by an infected udder during milking and not cleaned before utilization on an uninfected udder (Radostits et al., 2007).

1.2.6. Control and prevention measures for mastitis on SDF

Identifying factors associated with occurrence of mastitis is key to effective mastitis control and prevention protocols (Abebe et al., 2016; Karimuribo et al., 2006; Schepers & Dijkhuizen, 1991). It is also important to provide information about the economic impacts of mastitis in order to inform SDFs in low-income countries of the cost-benefit ratio of mastitis prevention/control measures (FAO, 2014). Economics is especially important for subclinical mastitis control and prevention, since its control relies on its identification and on a combination of other control measures (Ramírez et al., 2001).

Improving hygiene, both in the environment of the cow as well on the milking equipment, is paramount and is the first line of defense in the control of mastitis on SDFs (Moroni et al., 2018b; Reneau et al., 2005). The stall where cows lie down should be soft, dry and clean. Bedding should be changed frequently to prevent buildup of mastitis-causing bacteria. The design of the stall should be such that it allows drainage away from the stall. The alleyway should also be designed in such a way that it is able to be cleaned with ease, allowing water and urine to drain out of the shed. Recommendations for design have been explored previously and can be adopted to suit housing needs for cows in SDFs (Aleri et al., 2012; Kathambi, VanLeeuwen, Gitau, & Revie, 2019). Section 1.2.9 provides additional detail on these design features in SDFs.

Other management activities geared towards mastitis control have also been studied and found to be effective (Barkema et al., 2006a; Neave et al., 1969). These activities include: thorough washing of milking equipment after use; use of individual udder towels and ensuring teats are thoroughly washed and dried; proper milking techniques; and use of disinfectant teat dip. Prompt treatment of mastitic cows and milking them last reduces the chance of transferring the infection to other cows on the farm (Petzer et al., 2016). Culling cows with chronic intractable infections is helpful to remove the persistent source of infection at the farm. The role of farmer education has also been shown to be key in mastitis management and reducing the spread of AMR (Karzis et al., 2018).

Dry cow treatment is a good option for reducing the likelihood of a cow having mastitis at the start of the subsequent lactation. Dry cow treatment involves infusing a high dose of intramammary antibacterial preparation via the teats of a cow during dry-off. Blanket dry cow treatment involves treating all cows at dry off. In contrast, selective dry cow treatment can be administered to cows that have positive CMT and/or culture at dry-off. The latter strategy has been found to reduce prevalence of *S. aureus* significantly between dry-off and calving on SDF in Kenya (Sang et al., 2021) and has been promoted around the world to prevent antimicrobial resistance (McCubbin et al., 2022). This practice is, however, rarely practiced in SDFs and could be promoted by on-farm trials and training.

Mastitis vaccination is a field that is gaining popularity and attention as it tries to unlock better mastitis control benefits. Numerous studies have been done to explore vaccine development for major mastitis causing pathogens, including *S. aureus*, *E. coli*, and *Streptococcus* species, but their development and commercialization has been faced with several hurdles, including uncertainty on return-on-investment (Rainard et al., 2021, 2022). The *E. coli* vaccines, that have been developed and shown to reduce the severity of coliform mastitis (Ismail, 2017) have had low uptake by SDFs, demonstrating some disconnect between the knowledge, attitudes and practices of SDFs and possible gains from these mastitis vaccines. If mastitis vaccines were used on SDFs, vaccination could play a key role in reducing antibacterial use on SDFs and help reduce AMR.

1.2.7 Treatment options for mastitis on SDF

Mastitis is the most common reason for use of antibacterial medicine on dairy farms and as such, prudent use is recommended (Ruegg, 2017). Prompt treatment is among the strategies for effective control of mastitis. Teat infusion with antibacterial is the conventional mode of treating mastitis. Some of the intramammary preparations also include an anti-inflammatory medicine which aids in relieving pain and swelling in clinical mastitis cases. Cure rates are best when treatment is informed by laboratory culture and identification, and antibacterial sensitivity testing. However, *S. aureus* is known to form biofilms which help it evade treatment from intramammary infusion (Jones et al., 1998; Sears et al., 1990; Ziv & Storper, 1985). Hot compress use is also known to aid the cure of mastitis since it improves blood circulation in the mammary tissue, enhancing circulation of immune factors and medication to the infection site (Jean, 1997).

Antibacterial characteristics are important determinants in the effectiveness of mastitis treatment. Some antibacterials, such as tetracyclines, macrolides and trimethoprim-sulphonamides, are known to have somewhat reduced activity in milk (Fang & Pyörälä, 1996; Louhi et al., 1992). Bactericidal drugs are desirable for treating mastitis since phagocytic activity is limited in the mammary tissue (Kehrli & Harp, 2001). Selecting an antibacterial substance with low minimum inhibitory concentration is also important, especially if it is being administered parenterally due to possible intramammary penetration problems. For example, procaine penicillin G, being a weak acid, penetrates poorly into the mammary gland but due to the low MIC for susceptible organisms, therapeutic concentrations can be achieved in milk (Franklin et al., 1984; Ziv & Storper, 1985).

1.2.8 Antibacterial resistance patterns for common mastitis medicines on SDF

Successful antibacterial use has been threatened by antimicrobial resistance (AMR) arising from a variety of factors, including inappropriate dispensing and dosing, over use and self-prescription (Bebell & Muiru, 2014; Jasovský et al., 2016b; Padget et al., 2016). In a study conducted in Uganda, *S. aureus* isolated from milk samples of cows with subclinical mastitis showed multi-drug resistance (Kasozi et al., 2014). Recent studies are showing resistance to antibacterial drugs that were previously effective (Abdi et al., 2018).

Coagulase negative Staphylococci are more resistant to antibacterials than *S. aureus* and they easily develop multi-drug resistance (Sawant et al., 2009). However, studies have shown widespread resistance of *S. aureus* especially to procaine penicillin G (Olsen et al., 2006;

Ventola, 2015); thus, in vitro sensitivity testing of *S. aureus* to procaine penicillin G before treatment is recommended (Olsen et al., 2006).

Integrating mastitis control management and prudent treatment has shown to have an impact on reducing AMR (Karzis et al., 2018). However, a better understanding of the dynamics of AMR is required in order to save the world from the threat of increasing AMR.

1.2.9 Assessing stall design and management features for cow comfort and mastitis on SDF

Mastitis-causing bacteria access the udder usually from an ascending infection through a contaminated teat sphincter. The primary source of environmental contamination is when the cow lies down, and the udder is in contact with a dirty stall floor. Proper stall design coupled with good management protocols are important for a clean, dry, soft surface to lie down on, and with adequate space to be able to rise with ease. This management approach is key to reducing the occurrence of environmental mastitis in SDFs (Islam, Rahman, et al., 2012; Joshi & Gokhale, 2006).

There are a number of design features which aid in keeping the stall clean and dry, which reduces build-up of bacteria in the stall floor. Proper location of a neck rail and brisket board (Cook et al., 2005a) enhances a cow's ability to stand with its hind limbs at the edge of the stall and lie down with its rear end at the rear edge of the stall, leading to minimal urine and dung contaminating the stall. Comfort is enhanced when a cow is able to lie down on a dry soft surface and be able to rise with ease. This can be achieved by designing stalls in which the cows lie down optimally with minimal stall contamination with urine and dung. A stall with optimal width based on the cow's size ensures that the cow is not only able to lie down and rise with ease but also ensures it cannot turn inside the stall, thereby reducing chances of placing dung or urine inside the stall. The stall floor should be slightly slanted towards the rear to ensure easy drainage of the stall and should be without depressions where water can collect. Drainage and manure management around the stall also facilitates easy drainage of water and urine out of the shed so that it is not likely to flow into the stall and minimizes the cow carrying manure into the stall on its' hooves. An impermeable roof covering the whole stall will keep the stall dry from rain (Kathambi, VanLeeuwen, Gitau, & Revie, 2019).

1.2.10 Methods of assessing comfort in dairy cows on SDF

In order to improve comfort parameters for dairy cows, it is imperative that these parameters are understood well and measured to quantify the improvement needed and improvement made subsequent to the implementation of comfort recommendations. Various studies have explored the scope of comfort indicators (Bernardi et al., 2009; Cook et al., 2005a; Cook, 2009a; Dimov & Marinov, 2019; Keyserlingk et al., 2012). Lying time is a good indirect measure of comfort and has been explored widely in developed countries (Vasseur et al., 2012) and less so in developing countries (Kathambi et al.,, 2019). The latter study is among the few that have been published in peer-reviewed literature to explore lying down time as a measure of comfort in SDFs in the developing countries. In the study, lying down time was improved significantly by improving stall parameters, such as dimensions, stall floor softness and hygiene.

A different study explored how comfort can be objectively assessed by a knee test (McFarland, 1991) which assesses hardness and wetness of the stall floor. This test involves a person crouched in the stall, leaning forward so they are dropping on their knees, and describing the level of pain felt, as well as observing any wetness on their knees from the stall floor. This test mimics how a cow settles its body while lying down.

Hygiene can also be visually assessed both on the cow body and the stall floor. Dirty legs are an indicator of poor hygiene in both the stall and the alley way (Dimov & Marinov, 2019; Kathambi, VanLeeuwen, Gitau, & Revie, 2019; Lombard et al., 2010b). Studies have suggested methods of assessing hygiene by visual inspection - an assessment of upper leg cleanliness scored on a scale of 1-3 and on stall floor cleanliness scored on a scale of 1-5 was suggested by Reneau et al., (2005). A different scoring system has also been explored in Canadian dairy farms where upper leg cleanliness was scored from 1-4, reflecting good to poor hygiene (Dairy Farmers of Canada, 2021).

Finally, observations of the cow's body and the process it uses to stand up can also provide indicators of cow comfort. For example, the extent of any injury on the neck, carpi and hocks can indicate trauma on these body parts. Seeing the cow rise in the stall and noting if it does so with ease, and without striking its neck on parts of the stall structure while lunging and rising can identify stall design problems that may lead to a cow lying in an alleyway rather than in a stall, which is likely more contaminated than the stall.

1.2.11 Farm level compliance to mastitis control and cow comfort recommendations on SDF

In a randomized controlled trial (RCT) by Kathambi et al., (2019), SDFs in Kenya were given a set of stall design and management recommendations, and cow lying time, as an indirect measure of cow comfort, was assessed pre-and post-implementation of the recommendations. That study found a 75% compliance with recommendations given, and lying down time was significantly improved on intervention farms. The study, however, did not assess the benefits of improved comfort on occurrence of subclinical mastitis.

The animal welfare aspects of cow comfort and mastitis control are inherently important, but how cow comfort and mastitis control translate into reduced mastitis is particularly relevant to the health and productivity of SDFs. This information on reduced mastitis is important in order to guide farm-based decisions, as well as to inform policy on training needs of SDFs on cow comfort and mastitis control.

1.3 Thesis research objectives and structure

This study was conducted with the aim of reducing subclinical mastitis in SDFs through improved milking hygiene management coupled with stall design and management improvement. The ultimate goal of the research was to explore how SDFs can controlsubclinical mastitis through this integrated approach of mastitis control management and cow comfort improvement using minimal resources.

Specific objectives were as follows:

1. To measure occurrence of subclinical mastitis on SDFs, identify offending organisms through culture, and determine levels of antimicrobial resistance from positive-culture samples;

2. To assess mastitis control and cow comfort status in SDFs; and

3. To assess compliance with, and impacts of, cow comfort and mastitis control recommendations among SDFs.

The thesis is structured as follows:

- Chapter 1- An introduction of the research work, literature review and the study objectives

- Chapter 2 – A cross-sectional study to assess mastitis control and cow comfort status in SDFs. This chapter summarizes the baseline findings from the trial in Chapter 3 and 4.

- Chapter 3- A randomized controlled trial to assess compliance with cow comfort recommendations and their impact on improving cow comfort in SDFs.

- Chapter 4 – A randomized controlled trial to assess compliance with both cow comfort and mastitis control recommendations and their impact on subclinical mastitis on SDFs. Chapter 3 and 4 report results from the same randomized controlled trial (RCT) in Chapter 3, with Chapter 3 focused on the cow comfort recommendations and impacts, while Chapter 4 examines the combined effect of cow comfort and mastitis control recommendations on a different outcome – subclinical mastitis. Combining Chapters 3 and 4 would have led to one massive chapter that would have been difficult to publish in a peer-reviewed journal.

- Chapter 5 – A brief summary of the methods, findings and discussion from Chapter 2, 3 and 4, for those who do not have time to read through the full chapters. Conclusions and recommendations integrated from these three chapters are also summarized here.

Since SDFs are a major pillar of Kenya's economy and contributing to the livelihoods of millions of families, research on mastitis control efforts is needed in order to achieve sustainable development and maintain economic competitiveness in this sector. Research is especially needed to better understand how these common mastitis challenges facing SDFs can be controlled at minimal costs. Knowledge transfer is going to be essential to realize the gains from this research.

1.4 Study partners and background.

The research project was made possible by support from different partners. It was part of an ongoing program being implemented in Meru, Kenya, with the aim of empowering SDFs to enhance productivity and quality of life through research and extension/training. Previous projects within the program have yielded benefits in improved reproduction of dairy cows (Muraya et al., 2018), and improved feed management and fodder production (Makau et al., 2018; Richards et al., 2019). The project had substantial support from Farmers Helping Farmers (FHF), a Canadian non-profit organization working to support local dairy farmers. FHF has established an existing platform for entry into the community, where the data collection was facilitated from the existing FHF and UPEI relationship with the Naari and Buuri Dairy Farmers Cooperative Societies. These two dairies provided the project with logistical support by navigating the study team to the farms. The societies served as the primary contact point between the study team and the dairy farmers.

The primary funders of the project were Queen Elizabeth II Diamond Jubilee Scholarship Program (QES). QES scholarship funds are administered though Universities Canada, and the Rideau Hall Foundation, a Canadian non-for-profit organization that mobilizes resources, people and ideas towards sustainable and equitable development. The QES funding included some of the field work support for the MSc student while doing data collection in Kenya. The Sir James Dunn Animal Welfare Centre provided partial funding support for the field work as well, particularly focused on the cow comfort improvement on the SDFs in the study. The study was carried out in collaboration with the University of Nairobi that provided the laboratory for bacterial culture and susceptibility testing at the Department of Clinical Studies.

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Chapter 2: Cross-sectional study of cow comfort and management factors associated with subclinical mastitis in smallholder dairy farms in Kenya.

2.1 Abstract

A number of environmental and contagious factors have been associated with subclinical mastitis (SCM), which is a common and costly problem for smallholder dairy farmers (SDF). A cross-sectional study was conducted on 118 cows in their first two months in milk on 109 SDF in Kenya. The study objective was to investigate the relationships among various cow and farm management parameters and SCM specific to SDF.

The stall floor comfort level was assessed through knee impact and wetness tests, and cleanliness on the leg and udder were also scored. Various mastitis prevention measures were also assessed (e.g., milking protocols, and use of teat dip and dry cow therapy). Individual quarter SCM was assessed on each cow using California Mastitis Test (CMT). Univariable and multivariable logistic regression models were fit to determine management factors associated with cow-level SCM.

Farm-level, cow-level and quarter-level prevalence of SCM was 45.9% (50/109), 43.2% (51/118) and 21.9 % (103/471), respectively. The proportion of stalls scored as dirty was 33.1% while 49.1% of cows had dirty legs. Only 10.1% of farms were using either disinfectant teat dip or dry cow therapy (or both) to prevent mastitis. Low parity and poor stall hygiene were significantly associated with occurrence of SCM. At high daily milk yield, the probability of having SCM was higher in cows housed in a shed with a dirty versus clean alleyway, with no significant difference at low daily milk yield.

From the study findings, we concluded that certain cow characteristics and comfort measures were associated with SCM and need to be incorporated in education plans for farmers in SDF.

Key words: sub-clinical mastitis, smallholder dairy farms, cow comfort, stall hygiene, dairy cows.

2.2 Introduction

Mastitis is the most important cause of economic losses in the dairy industry around the world (Abebe et al., 2016; Bardhan, 2013) and is associated with milk reduction from infected quarters, treatment costs, milk rejection due to spoilage, and culling cows with recurring mastitis, among others. Mastitis is a painful disease that can be classified into clinical and subclinical, based on observable changes in the udder and milk, and categorized into environmental and contagious, based on the primary reservoir of the pathogens involved (Klaas & Zadoks, 2018; Smith et al., 1985). Subclinical mastitis (SCM) is considered the most economically important form of mastitis (Mungube et al., 2005) due to its higher prevalence, need for detection, and long-term effects, as compared to clinical mastitis. It accounts for more than 90% of total loss in milk production (Schepers & Dijkhuizen, 1991), and is a substantial animal welfare concern (Peters et al., 2015). CMT is a quick and reliable qualitative screening test and is an easy way to indirectly measure somatic cell count used widely to detect SCM (Leslie KE, Jansen JT, Lim GH, 2002).

The dairy industry is a strong pillar in the economy of many developing countries. In Kenya, 80% of the dairy cattle population is on two million smallholder farms, which contribute an estimated 60% of the country's milk supply (Bonilla et al., 2017; Peere & Omore, 2017), and 8% of the national gross domestic product (USAID & GoK, 2009). Previous studies have estimated cow-level prevalence of SCM in Kenya to be between 44% and 65% (Gitau et al., 1994; Muraya et al., 2018; Mureithi & Njuguna, 2016).

While rearing dairy cows on pasture is the natural environment, it is not possible on most SDF in central Kenya due to decreasing land sizes and tick-borne disease control recommendations (VanLeeuwen et al., 2012). For these reasons, a majority of SDF rear their cows in zero-grazing systems using confinement free-stall units, where a cow has a stall to lie down and a short alleyway to walk to a nearby feed and water trough (Gitau et al., 1994). If well-constructed and maintained, these structures can provide excellent comfort and welfare levels, promoting good hygiene and low mastitis incidence. Unfortunately, many SDF in developing countries have lagged in adopting practices and structures that promote optimal welfare of their dairy cows (Kawonga et al., 2012; Nkya et al., 2007) and thus, SDF continue to grapple with the associated milk production losses.

Design and stall maintenance have a major effect on cow comfort parameters. A cross-sectional study on 80 Kenyan SDF found skin abrasions on 85% of hocks, 75% of carpi, 61% of necks, 44% on briskets and 29% on udders and teats (Aleri et al., 2011; Aleri et al., 2012). Cows that frequently lie down in dirty stalls or alleys have poor hygiene scores, and subsequently, more mastitis (Sant'Anna & Paranhos da Costa, 2011).

Numerous studies in developed countries have shown associations between cow comfort outcomes and production indices, such as mastitis and milk production. (Lombard et al., 2010; Sprecher et al., 1997; Tucker et al., 2004; Zurbrigg et al., 2005) A previous study conducted in Kenya found significant association between stall comfort parameters and cow lying time (Kathambi et al., 2019) but did not explore the comfort effects on mastitis.

It is important to determine the current comfort status and practices in SDF, and particularly those that affect the occurrence of SCM, in order to guide informed interventions, and recommendations towards comfort standards. This study aimed at investigating the status and impact of cow comfort and mastitis management practices, on the occurrence of SCM in SDF in central Kenya.

2.3 Materials and methods

2.3.1 Ethical approval

This study was approved by the Research Ethics Board and the Animal Care Committee of the University of Prince Edward Island. We received consent from the Naari and Buuri Dairy Farmers Cooperative Societies, and Farmers Helping Farmers, a partnering non-governmental organization working with the dairy societies. Written consent was also sought from individual farmers on the first farm visit.

2.3.2 Study population.

The study was conducted in Buuri Constituency in Meru County in the central region of Kenya. Farmers in this region mainly practice mixed farming, whereby dairy farming is conducted alongside the cultivation of potatoes and other vegetables. Typical dairy units have less than 5 cows, with a majority having only one or 2 milking cows. An initial sampling frame of 1500 farms shipping milk to Naari and Buuri Dairy Farmers Cooperative Societies was provided, from which we recruited all farms that met the following criteria: 1) less than five milking cows; 2) cows reared in a zero-grazing unit; and 3) at least one cow that was less than 60 days in milk at the time of recruitment. To attain a 25% difference in SCM between factor positive and factor negative cows, with the desired power of at least 0.8 and 0.05 significance level, we needed a sample size of 116 cows. We recruited all farms that met the inclusion criteria during the two-month recruitment period and ended up with a total of 118 cows on 109 farms recruited.

2.3.3 Data collection

We conducted the study between August and October 2020 which is usually a drier period of the year. A questionnaire administered in-person, in the farmers' local dialect (Kimeru), was used to collect farm and animal demographic characteristics. Questions also included aspects of mastitis prevalence, cow comfort and mastitis management practices on the farm (e.g., bedding and manure management).

General health status (by routine veterinary physical examination) and body condition score (scored on 1-5 scale with ½ point increments) (Wildman et al., 1982) were assessed for each cow. Cow weight was estimated using a heart girth tape. Hygiene scoring of each cow's udder, flank and legs, and freestall hygiene was assessed, using a whole point scale of 1 (very clean) to 5 (very dirty) (Reneau et al., 2005). Injuries and lameness, scored from 1 (no injuries/ lameness) to 3 (severe injuries/lameness) were assessed, modified from a known 1-5 score (Sprecher et al., 1997), which combined score 2 and 3 together, as well as 4 and 5 together. Injuries were assessed at the neck, carpus and hock regions.

Each cow was tested for individual quarter subclinical mastitis using California Mastitis Test (CMT), whereby the first strip of foremilk was milked from each teat into separate wells of a CMT paddle. An equal amount of 3% CMT solution (Immucell Corporation, USA) was added to each milk sample and whirled for about 15 seconds. Color changes and consistency of the mixture were then observed and used as a diagnosis for presence and severity of SCM (Harmon, 1994). Quarter level subclinical mastitis was scored as negative (0 or trace) and positive (1, 2, or 3) with increasing severity of SCM (National Mastitis Council, 2004; Quinn et al., 1994).

Total length (front wall to rear curb), body length (neck rail to rear curb) and width of each stall were measured in centimeters and categorized as 1 (adequate), 2 (marginal), or 3 (inappropriate) based on recommendations relating to the weight of the cow lying in the stall (Cook, 2009). Based on its height from the floor of the stall and distance from the rear curb, neck rails and brisket boards were reported as: 1) present and well-positioned, 2) present but wrongly positioned, or 3) absent. Forward/side lunge space and leg space were reported as: 1) sufficient, 2) marginal, or 3) inappropriate, based on recommendations (Cook, 2009). Knee impact and wetness tests (McFarland, 1991) were used to assess the condition of the stall floor. Presence and type of bedding in the stall was also recorded. Alleyway hygiene was scored, based on the amount of manure present, as clean (no manure), fairly clean (small amount of manure that can be easily avoided while walking) and muddy (large amount of manure that could not be avoided while walking). The roof of the cow shed was examined for holes and for appropriate coverage of the cow stall. It was also recorded whether surface water was able to flow into the stalls or diverted around the stalls.

2.3.4 Statistical analysis

Information on the questionnaires was entered into Microsoft Excel (Microsoft lnc., Sacramento, California, USA) where it was cross-checked for accuracy and coded, and later imported into Stata 16.1 (Stata Corp LLC, College Station, Texas, USA) for statistical analysis. Injury scores were dichotomized as either 0 (no lesions) or 1 (scores 1 and above), since scores above 1 were few, and were reported as injury prevalence. Hygiene scores were also dichotomized, with scores 1 and 2 classified as 0 (clean) and scores 3, 4 and 5 (dirty) classified as 1. Cow-level prevalence of SCM was reported based on a cow having at least one quarter positive (score 1 and above) on CMT. Farm-level prevalence was reported based on farms that had at least one cow with at least one quarter positive on CMT. Quarter-level prevalence was reported as the proportion of all quarters sampled that tested positive on CMT.

Logistic regression modeling was utilized to determine associations between predictor variables and cow-level SCM (outcome variable). Parity was recategorized for improved model fit, where parities 2 and 3 were grouped together, and parities 4 and above were grouped together. Current daily milk yield was categorized into three groups: having milk yield between 1 and 8, 9 and 15, and above 15 liters per day, guided by a Lowess smoother plot of linearity between probability of having SCM and current daily milk yield. Predictor variables were initially assessed for univariable associations, and those with p-values equal to or below 0.25 were retained for multivariable regression. Correlation among variables that met this cut-off was determined using either Pearson correlation coefficient (continuous variables) or Chi-square test (categorical variables). Backward stepwise elimination was used to systematically remove variables with no significant association with SCM from the model. The causal diagram in Figure 1 was utilized to guide the model building. Each of the removed variables was individually fitted back into the final model to assess for uncontrolled confounding. Interaction was also assessed for all pairs of final model variables. Goodness of fit for the model was assessed using the Hosmer-Lemeshow test, and influential observations were assessed by evaluation of standardized Pearson residuals, leverage and delta beta. A mixed effect model was not explored since only 4 farms had more than one cow with three having 2 cows and one having 3.

2.4 Results

2.4.1 Descriptive statistics

The study population consisted of 118 cows from 109 SDF. The breeds consisted predominantly of Bos taurus dairy breeds (Friesians, Ayrshires, Guernseys and Jerseys; 87.3%) and their various crosses (11.0%) and a small portion of Bos indicus cows (1.7%). The body condition of the cows ranged from 1 to 3.5, with 65.3% having a body condition score of 2.5 and above. They weighed on average, 350 kg (±69.5 s.d.), ranging from 230 to 698 kg, and had an average daily milk yield of 10.7 (±4.28) liters, ranging from 1.5 to 28 liters. Only 11 of the farms (10.1%) used dry cow treatment and disinfectant teat dip, while 29 (26.6%) were familiar with California Mastitis Test (CMT) by virtue of having a cow on their farm previously checked for and diagnosed with subclinical mastitis.

Farm-level SCM prevalence was 45.9% (50/109). Cow-level prevalence of SCM was 43.2% (51/118). One cow had the right-fore quarter completely dried off due to a previous mastitis problem, leaving 471 quarters from the 118 cows. Quarter-level prevalence of SCM was 21.9% (103/471). Out of the 109 farms recruited, 35 (32.1%) had experienced a case of mastitis in at least one of their cows within the last one year.

The study cows were all reared in zero-grazing units, and 87.2% of farms (95/109) had freestalls with partitions between stalls. Among the 95 farms with separate stalls for individual cows, 38 (40%) had a neck rail, of which 18 were well-positioned and 20 were inappropriately positioned. Of these 95 farms with separate stalls, 11 (11.6%) had a brisket board, of which 3 and 8 farms had poorly and well-positioned brisket boards, respectively. Only 14 of the 109 farms (12.8%) lacked an appropriate cow shed roof (too short or holes), while surface water was observed leaking into the stalls in 19 (16.2%) of the sheds.

For the 118 cows, a majority of their stalls (111, 94.1%) had a dirt floor, while 5 (4.2%) had concrete floors and 2 (1.7%) had a wooden stall floor. Two-thirds of the 118 stalls (67.8%) had bedding in the stall. Regarding bedding types for the 118 cows, 31.4% had crop waste, 31.4% had wood shavings or saw dust, 2.5% had additional loose soil, and 0.8% was straw. The remaining 40 stalls had no bedding, although rubber mats were used for stalls on two of the farms.

On a scale of 1 (very clean) to 5 (very dirty), 16 (13.6%), 63 (53.4%), 31 (26.3%), 7 (5.9%) and 1 (0.9%) stalls were scored as 1, 2, 3, 4 and 5, respectively. When recategorized on a dichotomous scale (Table 1), 79 (66.9%) stalls were scored as clean (scores 1 and 2) on the day of the visit while 39 (33.1%) stalls were categorized as having a dirty floor surface (score 3, 4 or 5). In terms of hygiene, close to half of all cows had dirty legs (Table 1), while a fifth of udders were dirty. Alleyways were largely categorized as clean. The proportion of cows with neck lesions was substantially higher than for carpal and hock lesions (Table 2.1). Lameness was rare.

Farmers were applying a number of mastitis control protocols (Table 2.2). All farms were milking all their cows by hand. Most of the farmers were giving fresh feed after milking (87.0%), washing hands between milking different cows (80.7%), milking mastitic teats last (91.7%), and milking mastitic cows last (65.9%), where applicable. However, other mastitis control measures were infrequently employed, such as using different towels to wash each cow udder (49.5%), using dry cow therapy (10.1%), using disinfectant teat dip (10.1%), and stripping the first milk to assess physical changes suggestive of mastitis (21.1%).

2.4.2 Factors associated with occurrence of cow-level subclinical mastitis

Out of the factors assessed for unconditional association, parity, alleyway hygiene, stripping out first milk, having mastitis in the previous year, breed, body condition score, knee impact, current daily milk yield, and stall hygiene met the cutoff to be included in the multivariable regression model (Table 2.3). In the multivariable model, parity, alleyway hygiene, stall hygiene, current daily milk yield, and an interaction between alleyway hygiene and current daily milk yield had significant associations with occurrence of subclinical mastitis (Table 2.4). Only 4 out of 114 farms had more than 1 cow; one farm had 3 cows while the other three had 2 cows each. Herd level variability was therefore not explored. None of the removed variables were re-introduced to account for uncontrolled confounding.

Interpreting the coefficients in Table 2.4, cows in the second and third lactations were 3.4 times (i.e.,1/0.29) less likely to have SCM compared to cows in their first lactation, while those in their fourth and subsequent lactations were 8.3 times (i.e., 1/0.12) less likely to have SCM compared to cows in their first lactation. Cows that had a dirtier stall were 4 times more likely to have SCM. The coefficients of the dirty alleyway variable and daily milk production variable cannot be interpreted independently without considering the other variable, and Figure 2.2 demonstrates this dependency in the interaction variable involving these two variables. At low milk production, there was no difference in the odds of SCM by alleyway hygiene score; however, at higher milk production levels, the odds of SCM were lower with a clean alleyway score (score = 1 or 2) and higher with a dirty alleyway score (score = 3). There was considerable negative correlation (-0.37) between alleyway hygiene and stall hygiene, indicating that clean stalls were sometimes observed in sheds with dirty alleyways, and vice versa, Table 2.5.

The Hosmer-Lemeshow goodness-of-fit test showed good fit of the model to the data ($p=0.387$). There was only one standardized Pearson residual greater than 3 (3.18) and none less than -3. The greatest leverage value was 0.27.

2.5 Discussion

This study is the first to simultaneously explore and demonstrate the relationship between cow comfort and mastitis control parameters and occurrence of SCM in SDF in developing countries. Cow-level (43.2%) and quarter-level (21.9%) SCM prevalences were lower than reported in previous studies in Kenya. Others (Mureithi & Njuguna, 2016) found cow-level prevalence of 64% and quarter-level prevalence of 55.8%. Cow-level prevalences of 56% and 65% were found in two other districts in Kenya (Bundi et al., 2014). Our lower prevalence could be seasonal variation (the sampling time was at the end of the dry season), or it could be attributed to the farmer assistance and education program offered by Farmers Helping Farmers.

The proportion of farms with a partitioned stall (87.3%) is consistent with other studies conducted in Kenya (Aleri et al., 2012; Kathambi et al., 2019) which found 83% and 87.4%. The proportion of stalls reported as dirty (33.1%) and use of bedding in the stalls (68.4%) was consistent with other findings from studies done previously in the same region (Kathambi et al., 2019), which found 35% dirty stalls and 72% of farms using bedding on the stall floor. Our study found that cows that were lying down in stalls with poor hygiene were associated with more mastitis, an important association. We hypothesized an association between bedding and injuries and stall hygiene. This study, however, reported a low prevalence of injuries, so we didn’t model factors associated with injuries. Theoretically, stalls with good dry bedding would likely have good stall hygiene and fewer hock and knee injuries.

For the interaction between alleyway hygiene and current daily milk yield, at low milk production, there was no difference in the odds of SCM by alleyway hygiene score; however, at higher milk production levels, the odds of SCM were lower with a clean alleyway score. This finding demonstrates the importance of a clean-living environment for dairy cows, not just the stall. Cows will carry manure and mud on their feet from the alleyway into their stalls if the alleyway is left uncleaned, leading to udder exposure to manure and mud in the stall.

The negative correlation observed between alleyway and stall hygiene levels could be explained by farmers not cleaning the alleyway as often as the stall, or not cleaning the stall as often as the alleyway. This negative correlation could also suggest that some cows were not using their stalls and preferentially lying down in the alleyway due to a dirty or lumpy stall or a stall with inappropriate dimensions for the various rails used for the stall (e.g., short stall length). Cows that don't often lie down in the stall means that they don't pass manure or urine in the stall, so the stall remains clean. Cows lying down on a dirty alleyway have been found to be more likely to have higher incidence of mastitis (Kathambi et al., 2019; Kerro & Tareke, 2003; Lakew et al., 2009; Mungube et al., 2005) compared to those lying down in a clean stall. This result also greatly underpins the important interplay between proper stall design and management, potential animal welfare indicators and various mastitis control protocols in the effective management of udder infections in these farms.

The substantially more neck injuries observed in the study was noted to be contributed not only from the neck rail in the stall but also from the poles on top of the feed bunk preventing cows from entering it. The design of the feed bunk was noted to be an important contributor of injuries when the pole was placed too low and rubbing on the cow's neck. Therefore, feed bunk design should be given due consideration while designing zero-grazing units. Injuries on other body regions and lameness were minimal and substantially less than what was reported in previous studies (Aleri et al., 2011; Aleri et al., 2012). This disparity could be attributed to differences in scoring used in the two studies or due to actual differences in prevalence of injuries.

Our study found the average daily milk yield (10.7 kg/day) to be substantially higher than in a study conducted in the same locality previously (Kathambi et al., 2018) which reported 6.6 kg/day, and slightly higher than the 9.3 kg/day reported in a study done in the Mukurweini district of Kenya (Richards et al., 2019). The latter study recruited recently calved cows, which was similar to our study population. Conversely, the former study recruited at the herd-level, and they were not specific about recruiting cows in a specific lactation stage (Kathambi et al., 2019), while we recruited cows in their first 2 months post-calving, which is associated with a peak in milk production. Also, the difference in milk production between our study and the former study could be attributed to a continuing education program working with dairy farmers in the region to equip them with knowledge on better husbandry, feeding and breeding protocols.

Increasing parity was associated with decreasing odds of having mastitis. This was contrary to studies conducted elsewhere (Islam, Rahman et al., 2012; Islam, Islam et al., 2012; Joshi & Gokhale, 2006; Kerro & Tareke, 2003; Nibret & Tekle, 2012). An increase of mastitis incidence with parity was attributed to an increased immunologic reaction of teats to infections and increased degree and frequency of previous exposure (Lakew et al., 2009). Our conflicting findings could have been driven by other factors, such as the level of mastitis management and control measures being applied on different farms. Farms that were implementing more mastitis control protocols are likely to be more informed about general husbandry; and thus, possibly taking better care of their animals. Consequently, they are likely to keep their cows longer compared to farms with mastitis problems, among other production-related challenges, which may have led to culling their mastitic cows at an earlier age. Previous cultures of mastitis in Kenya have shown substantial infections with *S. aureus*, which is often refractory to treatment, making culling an option to consider (Bundi et al., 2014).

We expected to find significantly fewer cases of SCM in cows that were using dry cow therapy and teat dip disinfectant. However, the farms that applied these protocols were few, and so we could not detect significant associations to these factors. The low use of these management tools can be attributed to low knowledge levels, since a majority of the farmers that were not using these products reported that they were not aware that such products existed.

Regarding study limitations, some measures, such as stall and alleyway hygiene, were done subjectively by the principal investigator and an assistant. The study utilized a scoring system described by Reneau (Reneau et al., 2005) which outlined a scoring system for cow body hygiene but modified the scoring to score stalls and alleys, although there were no particular cut points to reference. The validity of this scoring system was enhanced by regular cross-checking between the principal investigator and the assistant by referring back to a diagram with the various score points and scoring together to ensure consistency in the assessments.

Being cross-sectional in nature, results from the study are not reliable to make a causal inference about the outcome from the predictors, as there is no element of temporality between the two. The model predictor variables were assessed at the same time as the outcome, making it impossible to confirm that they existed prior to the outcome. The study was also prone to recall bias, with farms that had their cows treated for mastitis previously likely being more conversant with routine mastitis control protocols that were advised by the veterinarian that treated their cows. Since most of the farmers had low-to-moderate knowledge levels in mastitis control, such farmers with a previous encounter with mastitis could still be practicing most of the protocols advised by the veterinarian, compared to farmers that had not had a cow treated for mastitis on their farms.

Since a harmonized scoring system for overall cow comfort does not exist, we used different components of cow comfort to build the model. As such it was not possible to precisely assess the relationship between overall comfort of each cow and subclinical mastitis. There is need for a harmonized scoring system for cow comfort to be used as a standard in studies such as this one that seeks to relate cow comfort to other factors impacting on the wellbeing of dairy cows. Lessons can be borrowed from the body condition scoring of dairy cows (Wildman et al., 1982) which utilizes the status of different regions of the body of the cow. It would be of great benefit if a cohort or randomized controlled trial could be conducted to validate the outcomes of this study and establish a causal relationship between the factors incriminated in the occurrence of SCM in this study. For example, there is need to further explore the relationship between stall hygiene, alleyway hygiene and occurrence of SCM on SDF in Kenya, and the different factors that may influence these relationships.

2.6 Conclusion

Subclinical mastitis remains highly prevalent among Kenyan SDFs. There is relatively low uptake of some important routine mastitis control measures. Poor hygiene of the alleyway and stall were important factors associated with SCM and are highly dependent on stall design and management. Low knowledge levels appear to account for much of why these recommended practices have been poorly adopted, therefore more education on best management practices around cow housing and routine mastitis management protocols is needed.

2.7 Declaration of interest

The authors declared they had no competing interest in the study.

2.8 Acknowledgments

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Table 2. 1: Prevalence of poor hygiene and injuries observed on 118 cows on 109 smallholder dairy farms in Kenya, August to September 2020.

Outcome	No. of cows	Prevalence(%)	95% CI
Stall hygiene[A]	39	33.1	24.6 – 42.3
Leg hygiene[A]	58	49.1	39.3 – 58.5
Udder hygiene[A]	23	19.5	12.8 – 27.8
Alleyway hygiene[A]	97	82.2	74.1 – 88.6
Neck injuries	22	18.6	12.1 – 26.8
Carpal injuries	4	3.39	0.93 – 8.45
Hock injuries	3	2.54	0.52 – 7.19
Lameness	1	0.85	0.02 – 4.63

[A]Number/ prevalence of hygiene categorized as dirty (≥ 3 on a 1-5 scale)

Table 2. 2: Prevalence of farms that practiced various mastitis management protocols among 109 smallholder dairy farms in Kenya.

Outcome	No. farms responded	No of farms applying	Prevalence (%)	95% CI
Give fresh feed after milking	108	94	87.0	79.2 – 92.7
Wash hands between cows[A]	89 [A]	68	76.4	66.1 – 84.8
Mastitic teat milked last[B]	48 [B]	41	85.4	72.2 – 93.9
Mastitic cow milked last[C]	39 [C]	30	76.9	60.7 – 88.9
Different udder towels[D]	80 [D]	54	49.5	39.8 – 59.3
Disinfectant teat dip	109	11	10.1	5.15 – 17.3
Dry cow therapy (antibiotic)	109	11	10.1	5.15 – 17.3
Strip out first milk	109	23	21.1	13.9 – 30.0

[A] only includes farms that had multiple milking cows.

[B] only includes farms that had encountered mastitis.

[C] only includes farms that had more than one milking cow and had experienced mastitis in the past.

[D] only includes farms that had multiple milking cows.

Table 2. 3: Factors associated with occurrence of subclinical mastitis in univariable analyses

Factors	Categories	No. cows	Odds ratio	Odd ratio 95% CI	P-value
Parity	Reference	39			0.065[A]
	2 and 3	49	0.58	0.25 – 1.35	0.208
	>=4	29	0.29	0.10 – 0.83	0.020
Alleyway hygiene	Clean	21			
	Dirty	97	3.48	1.15 – 10.5	0.054
Strips out first milk	No	93		0.16 – 1.13	
	Yes	25	0.43		0.089
Mastitis in last year	No	81			
	Yes	37	2.63	1.18 – 5.48	0.047
Breed	Other breeds	46			
	Friesian	72	2.07	0.96 – 4.46	0.065
Body score condition	>=2.5	77			

	<2.5	41	0.48	0.21 – 1.05	0.068
Knee impact	Reference	46			0.250[A]
	2	61	0.52	0.24 – 1.13	0.097
	3	11	0.76	0.20 – 2.86	0.689
Current daily milk yield (L)	<8	28			<0.001[A]
	8-15	70	0.79	0.32 – 1.92	0.600
	>15	19	2.89	0.41 – 9.81	0.080
Stall hygiene	<=2	79			
	>2	39	1.63	0.75 – 3.53	0.216

[A]Global p-value for categorical variable

Table 2. 4: Factors associated with occurrence of sub clinical mastitis and their significance in a multivariable logistic regression model among 109 smallholder dairy farms in Kenya.

Factor	**Categories**	**No. cows**	**Odds ratio**	**95% CI**	**P-value**
Parity	1	39			0.005[A]
	2 & 3	49	0.29	0.10 – 0.82	0.020
	> 3	29	0.12	0.03 – 0.45	0.002
Stall hygiene	1&2	78			
	>2	39	3.99	1.36 – 11.7	0.012
Alleyway hygiene	1&2	20			
	>2	97	0.16	0.013 – 1.86	0.143
Current daily milk yield (L)	1-8	28			<0.001[A]
	8-15	70	0.02	<0.01 – 0.34	0.008
	>15	19	21.3	3.96 – 114.2	<0.001
Alleyway hygiene * Current daily milk yield Interaction term	B	B	B	B	0.003

[A]Global p-value for categorical variable

[B]Outcomes not reported for the interaction since interpretation relies on the main effects.

Table 2. 5: Pairwise correlation matrix for variables that had significant unconditional association with subclinical mastitis

	Parity	Alleyway hygiene	Strips out first milk	Mastitis in the last year	Breed	Body condition	Knee impact	Current daily	Stall hygiene
Parity	1.00								
Alley way hygiene	0.028	1.00							
Strips out first milk	-0.074	-0.030	1.00						
Mastitis in the last year	-0.167	0.019	0.069	1.00					
Breed	-0.208	0.084	-0.053	0.057	1.00				
Body condition score	0.055	0.013	-0.074	0.019	0.074	1.00			
Knee impact	-0.018	-0.184	0.113	-0.091	0.156	-0.024	1.00		
Current daily milk yield	0.184	0.052	-0.102	0.057	0.322	-0.120	-0.123	1.00	
Stall hygiene	0.088	-0.272	0.112	0.153	0.031	-0.110	0.034	-0.159	1.00

Figure 2. 1 Causal diagram showing association between cow and management factors and subclinical mastitis

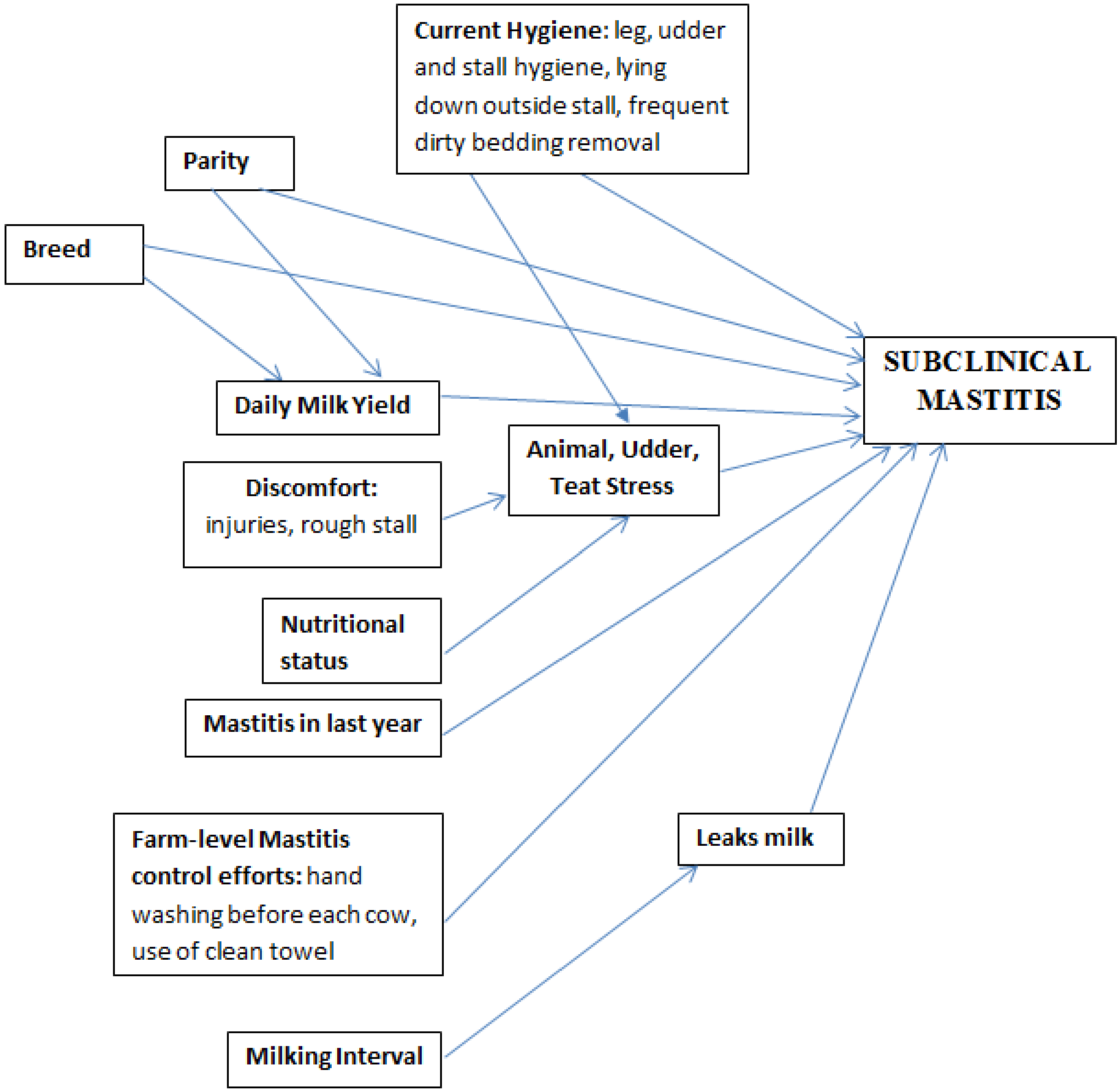
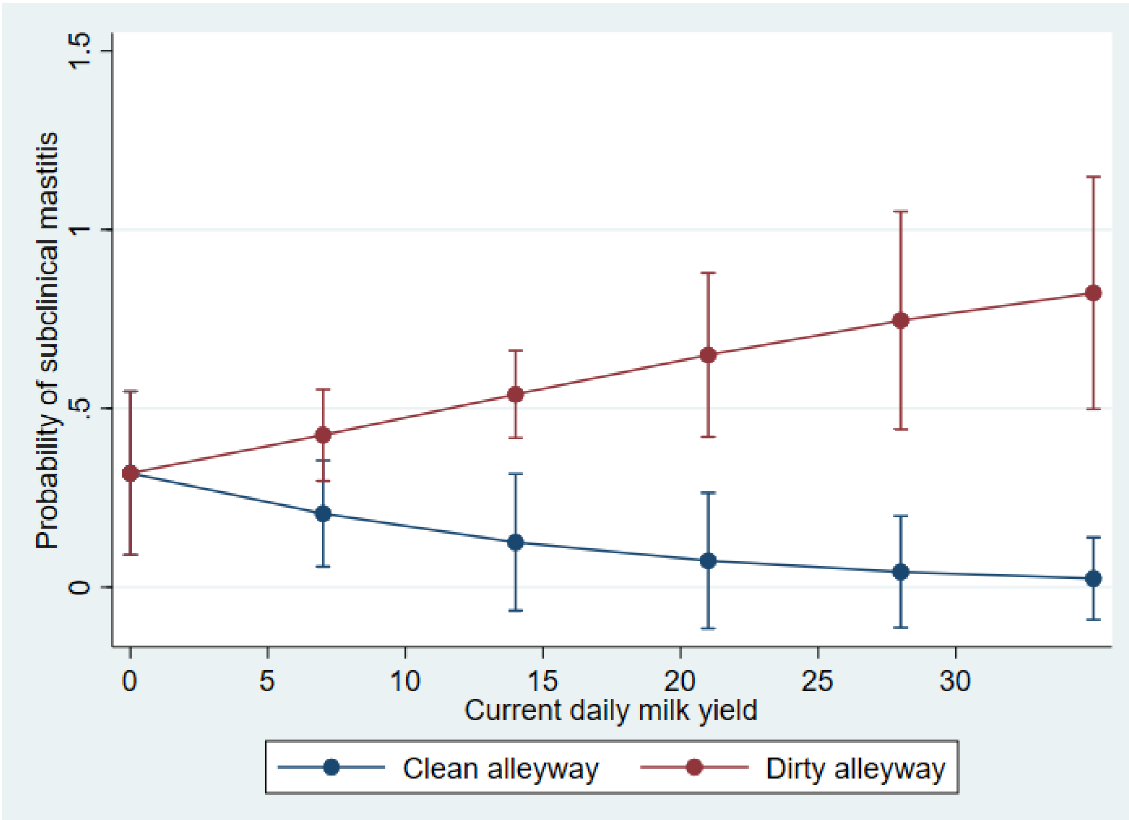


Figure 2. 2: Predictive margins plot of interaction between alley way hygiene and current daily milk yield among 118 cows on 109 smallholder dairy farms in Kenya



Chapter 3: Randomized controlled trial to investigate compliance with, and impacts of, cow comfort recommendations on smallholder dairy farms in Kenya

3.1 Abstract

Smallholder dairy farmers (SDF) in developing countries often have limited understanding of the importance of cow comfort. We conducted a randomized controlled trial with 124 cows on 114 Kenyan SDF to determine the status of cow comfort, to assess compliance to farm-specific cow comfort recommendations, and to evaluate the impacts of the farm-specific interventions on cow comfort. On the first farm visit, stall dimensions and characteristics (e.g. stall base hardness and hygiene) were measured and categorized as adequate, marginal or inadequate/absent based on cow size. Where measurements were not adequate, farm-specific cow comfort recommendations were provided in written and oral form to the randomly allocated intervention group of farms (n=74). On the second farm visit two months later, the same measurements were taken, and percent compliance to the recommendations was evaluated.

A discomfort index was arithmetically calculated based on the stall base hardness (scale was 1-3 for soft to hard) and hygiene (scale was 1-5 for clean to dirty). Multivariable linear regression models were used to determine specific associations with the discomfort index. On the first visit, the mean stall base hardness and stall hygiene scores were 1.7 and 2.3, respectively, for a mean discomfort index of 4.0. Intervention farmers were given 3.9 comfort recommendations, on average, and complied with 2.1 recommendations, significantly improving the discomfort index at visit two to 3.3. The overall compliance to the recommendations was 49.0%. In a final model, the interaction between intervention group and visit number was significantly associated with discomfort index, indicating that after adjusting for baseline

discomfort indices, the intervention led to better cow comfort. Specifically, bedding type and neck rail positioning were significantly associated with discomfort index. We concluded that farmers can substantially improve cow comfort on SDF by providing recommendations to them. Farm advisors should include cow comfort recommendations to SDF.

Key words: cow comfort, smallholder dairy farms, recommendations, compliance

3.2 Introduction

Animal welfare is increasingly becoming an important consideration in production animals (Madzingira, 2018). Various aspects of husbandry have been explored in order to achieve desirable welfare standards, especially around design, maintenance and management of animal housing (Cook, Bennett, & Nordlund, 2005a; Cook, Nigel B., 2009; Dimov & Marinov, 2019; von Keyserlingk et al., 2012). Fraser et al (1997) has described good animal welfare as the situation where the animal is functioning well, feeling well, and is able to live a reasonably natural life. The dairy industry worldwide has made remarkable progress on cow comfort (von Keyserlingk et al., 2012), realized more in developed countries.

In many developing countries, smallholder dairy farms (SDFs) are the primary source of milk production (Bonilla et al., 2017; Peere & Omore, 2017), providing nutrition and a source of income to rural families (Randolph et al., 2007). Most cows on SDFs in Kenya are housed in zero-grazing free-stall housing where they have a stall to lie down in, and a nearby feed trough where they can walk to and feed or drink water (Gitau et al., 1994). Most of these housing structures are wooden, with an iron-sheet roofing above the stalls, and less frequently also above the manger and drinking trough. Stall floors are usually dirt, with some made of concrete, with or without bedding or a rubber mattress (Kathambi et al., 2018).

Proper design and management of stalls is important for cow comfort and animal welfare (Fraser et al., 1997). Good cow comfort will likely also lead to enhanced productivity and possibly less diseases, such as mastitis, lameness and injuries (Dimov & Marinov, 2019). Unfortunately, cattle on SDFs are often kept in sub-optimal free-stall conditions (Kawonga et al., 2012; Nkya et al., 2007) partly because of insufficient resources, but also due to farmers' lack of knowledge about appropriate stall design and management, and cow comfort (Aleri et al., 2011; Aleri et al., 2012; Nkya et al., 2007).

Various methods have been employed to improve comfort for free-stall housed cows, especially in the stalls where they lie down. These methods include providing appropriate dimensions of the stall to match a cow's size, in both weight and height, adequate roofing, appropriate bedding on the stall floor, properly placed neck rails and brisket boards, providing adequate leg and lunge space, and ensuring good drainage around the stall (Cook et al., 2005; Cook, 2009; Tucker, et al., 2004). The presence of a properly placed neck rail and brisket board is known to enhance stall hygiene by preventing cows from urinating or defecating in the stall (Lombard et al., 2010).

In an earlier trial on SDFs in Kenya, lying time was shown to improve somewhat from recommendations given to SDFs (Kathambi et al., 2019). However, lying time is an indirect measure of cow comfort, whereas stall hygiene and hardness are more direct measures of cow comfort.

This trial aimed to: 1) assess compliance with cow comfort recommendations on stall design and management; 2) investigate factors associated with this compliance; and 3) assess the impact of compliance with these recommendations on direct measures of cow comfort of stalls on SDFs.

3.3 Materials and Methods

3.3.1 Ethical approval

The study was approved by the Research Ethics Board and Animal Care Committee of the University of Prince Edward Island, as well as from The Sir James Dunn Animal Welfare Centre, which partly funded it. We also received consent from Naari and Buuri Dairy Farmers Cooperative Societies and Farmers Helping Farmers, a partnering non-governmental organization that works with the two dairy cooperatives. Written consent was obtained from individual farmers during the first visit.

3.3.2 Study area, population and design

The study was conducted between August and November 2020 in Buuri sub-county of Meru County in central Kenya. Meru is 290 km northwest of Kenya's capital, Nairobi. It is at an elevation of 1930 m above sea level, at latitude 0.14 ^{o} North and longitude 37.57^{o} East. It receives an average annual rainfall of 1800 mm. Most residents in this region are farmers, most of them practising both crop and livestock farming.

We purposively recruited farms with cows that were 2 months or less in milk, from farms that were shipping milk to Naari and Buuri Dairy Farmers Cooperative Societies. In total, there are close to 1500 farmers with memberships to these two dairies, usually with 1-4 cows per farm. This study was part of another study working on mastitis; hence the desire to recruit cows in early lactation. For farms to qualify for the study as a SDF, they also had to have a zero-grazing unit, and have no more than five dry or milking dairy cows at the time of the study. We recruited all farms in these dairy groups that met the inclusion criteria within the period of the study.

The study was a randomized controlled trial carried out on 114 dairy farms between August and November 2020. A total of 78 cows on 74 farms were block randomly allocated to an intervention group while 46 cows on 40 farms being block randomly allocated to a control group. A coin was tossed by the first author for heads or tails (intervention or control) to determine the first farm status, with alternating blocks of farms afterward. Regarding our blocking variable, we avoided allocating farms that were very close to each other to different study groups, such as extended family members living within the same compound, to minimize bias arising from social interaction between intervention and control farms, which has a potential for contamination between study groups (Kathambi et al., 2019). More farms were allocated to the intervention group purposely (~2:1), based on guidance from a comfort compliance study on Kenyan SDFs by Kathambi et al., (2019) which found a compliance level of 66%.

3.3.3 Data collection

Data were collected on two farm visits, 2 months apart, between August and December 2020. On the first farm visit, farmers' demographic details, and farm-level stall management and husbandry data were collected by the first author using a semi-open-ended questionnaire administered to the principal farmer, where s/he was present, or the animal manager where the principal farmer was not available. An assistant was present to interpret it to their local language, where needed (Kimeru versus Kiswahili)

On both visits, various cow parameters were measured or observed from the cow by the first author and his assistant. Body condition was scored from 1 (very thin) to 5 (obese), with half point increments (Wildman et al., 1982). Weight was measured using a heart girth tape and height was measured via a tape measure at the height of the withers, using a level placed horizontally on the withers for precise measurement. Cow hygiene scores for the legs and udders were assessed from 1 (very clean) to 3 (very dirty) (Reneau et al., 2005).

Individual cow stalls were measured (Table 1) for dimensions (length and width), presence and placement of a neck rail and brisket board relative to the back of the stall, leg space (space between the stall floor and the lowest board between stalls) and lunge space (head space in front of the stall body length or between side rails). Stall hygiene was assessed on a scale of 1 (very clean) to 5 (very dirty) (Reneau et al., 2005), and stall wetness and hardness were measured using the knee test (McFarland, 1991). Injuries on the neck, carpi and hocks were assessed visually and scored as absent (0), mild (1) or severe (2) (Dairy Farmers of Canada, 2021) . Lameness was also visually assessed as the cow naturally walked and was scored on a 5-point scale (Sprecher et al., 1997) which was then condensed to a 3-point scale by collapsing 1 and 2 as well as 4 and 5 (1 to 3, absent to severe, respectively). The presence of an adequate roof above the stall was assessed based on coverage over the stall and the presence/absence of leaking holes. The stall parameters were categorized as adequate, marginal or inadequate/absent based on cow size (Cook, 2009; Tucker et al., 2004).

3.3.4 Intervention and compliance

A total of 12 recommendations shown in Table 1 had been previously identified as being important and appropriate for SDF sheds (Kathambi et al., 2019). Intervention farms were given specific recommendations from this list customized to their farms, depending on the areas that needed improvement (not adequate), as assessed by the first author. There was a variable number of recommendations given per farm.

Instructions were given to the farmers in their local Kimeru dialect orally and in written format. Changes that needed some construction were either drawn on paper with clear illustrations or were marked in the shed, with demonstration to the farmer in the shed. Recommendations were recorded as either major (score of 2) or minor (score of 1), depending on how much construction was needed to bring each of them to a desirable level. For example, moving a rail was considered minor, while adding length to a stall was considered major. Major recommendations carried more weight than the minor ones at the time of assessment for compliance, as explained below.

On the second farm visit, compliance with the recommendations was evaluated based on the measurements made on both visits. Item compliance for each recommendation was recorded as none, partial or full, depending on how well farmers executed each recommendation given. Full item compliance was assigned if the recommendation was implemented fully and correctly (score of 2), while no item compliance was assigned if the recommendation was not implemented (score of 0). Partial item compliance (score of 1) was assigned in the case of farms that attempted to implement the recommendations given but did not achieve the desired outcome. For example, one farmer tried to place a neck rail, but did not follow the actual dimensions so that the cow would strike the neck rail while rising.

3.3.5 Data handling and descriptive statistical analysis

Data were coded and entered into Microsoft Excel 2010 (Microsoft lnc. Sacramento, California, USA) where they were cross-checked for data errors, and preliminary descriptive statistics were conducted. The data were then imported into Stata 16.1 (Stat Corp LLC, College station, Texas, USA) for further descriptive and inferential statistical analysis.

A farm compliance score ranging from 0% to 100% was calculated for each farm. The numerator for the farm compliance score was the sum of all the major/minor scores of recommendations given multiplied by the full/partial/none item compliance scores for each recommendation for the farm. The denominator for the farm compliance score was the sum of all the major/minor scores of recommendations given multiplied by the full item compliance score, indicating the maximum possible item compliances for all the recommendations on the farm. The numerator divided by the denominator gave an overall percentage farm compliance score for each intervention farm. For farm aspects where no recommendation was needed, the major/minor score was 0, making that item not count in the farm compliance score for that farm. Full compliance of major recommendations had a substantial effect on the final farm compliance score, while partial compliance of minor recommendations had a small effect. An overall farm compliance score for the study was calculated as the mean of all individual farm compliance scores.

During data analysis, we arithmetically combined stall floor hygiene with stall floor hardness to create a discomfort index as a summary index of stall floor cow comfort. Type of bedding was recategorized into three categories in which no bedding, dirt or soil bedding, and rubber mattress were in one category, crop waste and straw were in another category, and sawdust and wood shavings was the third category. The presence of, and positioning of, a neck rail was also regrouped to a dichotomous variable to indicate either the presence of a suitably positioned neck rail or otherwise (i.e. no neck rail or a poorly positioned neck rail).

3.3.6 Analytical statistics

Univariable and multivariable mixed effect logistic regression models were used to determine factors associated with compliance of individual recommendations, accounting for clustering of recommendations within farms (objective #2). Those predictor variables that were univariably significant ($p<0.25$) with compliance were eligible for manual forward stepwise multivariable modeling.

To determine if the intervention was successful or not, univariable and multivariable linear regression models were used to determine associations with the discomfort index as the outcome. The discomfort index was right skewed; therefore, for appropriate model fit, the discomfort index was log-transformed, leading to a normal distribution Those predictor variables that were univariably significant ($p<0.25$) with log of discomfort index were eligible for manual forward stepwise multivariable modeling. The multivariable model attempted to include an interaction between study group and visit number to confirm the impact of the intervention on the second visit while accounting for the baseline discomfort index on the first visit. We also looked for other interactions between significant model variables.

We initially decided to compare discomfort index scores for the randomly allocated groups using the "intent-to-treat" principle – study groups remain as they were allocated. However, farms allocated to the intervention group that did not comply with any recommendations or complied minimally with their recommendations, would clearly lead to an underestimation of the beneficial effects of the intervention. Therefore, we explored a number of study group scenarios to accommodate for this underestimation, producing an "effective intervention group" and a "comparison group", as described below. For scenario one, those farms that did not have farm compliance scores of at least 40% were added to the control group to make the comparison study group, with the rationale for this 40% cutoff being explained in the results and discussion. The remaining allocated intervention group farms became the effective intervention group for this scenario.

For scenario two, the effective intervention group included those farms complying (at least partially) with at least one of the given recommendations (i.e. compliance score > 0%), while allocated intervention farms with 0% compliance were added to the control group for the comparison group, as done by Kathambi et al. (2019). For scenario three, the effective intervention group included only those farms having compliance of at least 40%, farms with compliance above 0% but below 40% were removed, and 0% compliance farms were combined with control farms for the comparison group, using the “per protocol” principle. Scenario one was the main scenario of interest utilized in the study because it retained the most farms and cows in the statistical analysis, while incorporating the natural break in the data shown in the results.

Variables that were not in the final multivariable models were individually added back into the models to evaluate confounding of 20% changes in the model coefficients (Dohoo et al., 2009). Goodness-of-fit of the discomfort index model was assessed visually by evaluating linearity of standardized residuals using a scatter plot of fitted values and standardized residuals, as well as statistically by the Shapiro-Wilks test for normality and the Breusch-Pagan test for heteroskedasticity. Models were also assessed for influential observations by evaluating leverage and Cook’s distance. Goodness-of-fit for the compliance model was assessed using Pearson’s Goodness of Fit Test.

3.4 Results

We recruited 124 cows from 114 smallholder dairy farms. There were 5 cows on 4 farms that were lost to follow-up after being sold from the farms, all from the intervention group (Figure 1). Therefore, the final study population was 73 cows on 70 farms in the allocated intervention group, and 46 cows on 40 farms in the allocated control group.

3.4.1 Farm- and cow-level descriptive statistics at visit one

The study cows consisted of various breeds, including Friesians (59.7%), Ayrshires (18.6%), Guernsey (7.3%), Jerseys (1.6%) and their various crosses (11.3%), and a small proportion of indigenous cows (1.7%). On the first visit, the body condition of these cows ranged from 1.0 to 3.5, with 65.3% having a body condition score of 2.5 and above. They weighed, on average, 350 kg (±69.5 s.d.), ranging from 230 – 698 kg, and had an average daily milk yield of 10.7 liters (±4.28), ranging from 1.5 to 28 liters.

The cows were reared on zero-grazing farms in which 87.4% (104/119) had individual partitioned stalls to lie down in, while the rest had an open shed without partitioned sleeping spaces for each cow. Among the 104 separated stalls, 42 (40.4%) had a neck rail, of which 20 (47.6%) were well-positioned and 22 were wrongly positioned. Eleven stalls (10.6%) had a brisket board, 8 (72.7%) of which were appropriately placed. For the 119 cows, 15 (12.6%) lacked an appropriate roof (too short or holes), while poor drainage of water around the stalls was observed in 19 (16.0%) of the stalls.

Most stalls (94.1%) had an earthen floor, while 5 (4.2%) had concrete and 2 (1.7%) had a wooden floor. Two-thirds of the 119 cows (67.2%) had bedding in the stall. By bedding types, 31.1% was crop waste, 31.9% was wood shavings or sawdust, 2.5% was loose soil, 0.8% was a rubber mattress, and 0.8% was straw.

On a scale of 1 (very clean) to 5 (very dirty), 17 (14.3%), 63 (52.9%), 31 (26.1%), 7 (5.9%) and 1 (0.8%) stalls were scored as hygiene 1, 2, 3, 4 and 5, respectively. When recategorized on a dichotomous scale (Table 2), 80 (67.2%) stalls were scored as clean (scores 1 and 2) while 39 (32.8%) stalls were categorized as having a dirty floor surface (score 3, 4 or 5). In terms of hygiene, close to half of all cows had dirty legs (Table 3) while a fifth of udders were dirty. The proportions of cows with carpal and hock lesions were low compared to neck lesions.

Overall, the discomfort index ranged from 2-7 with a median of 4 and a mean of 4.0 at the baseline visit. The mean discomfort index on visit 1 was 3.9 and 4.0 for the allocated intervention and allocated control groups, respectively (p=0.303). There were also no significant differences in hygiene or injury proportions between groups at the first visit (only lameness proportions were different between groups), indicating that the random allocation process was moderately effective at making the two groups equal at the start of the trial (Table 3).

3.4.2 Description of comfort recommendations given and respective compliance

Recommendations given to the farmers are shown in Table 1. Of all comfort recommendations given, fixing a neck rail was given to the most farms (82.4%), while fixing sharps was given to only one farm (1.4%), and that farm did comply (Table 2). Improving stall hygiene (83.3%), creating ample leg space (76.9%) and making the stall soft and dry (76.3%) accounted for recommendations with the highest item compliance scores. Four farms were given recommendations to fix the roof and two farms were advised to make some changes to the total length of the stall, but these farms did not attempt to make those major changes.

On the second visit, the overall mean farm compliance score was 49.0% among all farms allocated to the intervention group, but there was a natural break in farm compliance scores, with very few farms having a farm compliance score of approximately 40% (Figure 2). Among the 70 allocated intervention farms that had a second visit, 43 farms had a farm compliance score of at least 40% while 27 farms had substantially lower farm compliance scores (Figure 2). The mean farm compliance score among farms with 40% or more farm compliance was 75.1%.

3.4.3 Farm and cow level descriptive statistics at visit 2

Table 4 provides the proportions of various comfort parameters on the second visit, by group, for Scenario one, the main scenario of interest. All hygiene scores, the knee impact score, and proportion of lame, and carpal and hock injuries were numerically lower in the intervention group than the comparison group. While there were no significant differences in stall hygiene or knee impact scores between groups during the baseline visit (Table 3), there was a significant difference in stall hygiene ($p=0.012$) and knee impact scores ($p<0.001$) between groups for the second visit (Table 4), with the effective intervention group improving their scores compared to the comparison group.

Post-intervention, the overall discomfort index again ranged between 2 and 8, with a median of 3 and a mean of 3.7, and group means of 3.3 and 4.0 for the effective intervention group and comparison group, respectively. There was a significant difference between these study group means ($p=0.003$).

When comparing baseline and second visit stall hygiene (Table 3 and 4), there was significant stall hygiene improvement in the effective intervention group (a 17.8% decrease in proportion of stalls with poor hygiene), while the proportion of stalls with poor hygiene in the comparison group increased by 11.7% between visits. Similarly, the proportion of stalls with poor knee impact improved in the effective intervention group between visits (51.1% with poor knee impact versus 24.4%), while the proportion of stalls with poor knee impact in the comparison group only decreased from 67.6% to 58.1% between visits.

3.4.4 Factors associated with the discomfort index

Using scenario one as the scenario of interest, the following variables had significant univariable associations ($p \leq 0.25$) with the discomfort index; visit number, study group, type of bedding, neck rail positioning, and current daily milk yield (Table 3.5). In the final multivariable linear regression model (Table 3.6), bedding type, neck rail positioning, and current daily milk yield were significantly associated with discomfort index ($p \leq 0.05$). Interaction between study group and visit number was not significant in the final model. There was no confounding detected from variables dropped from the multivariable regression model.

While controlling for effects of other variables in the final model, and exponentiated coefficients, using crop waste and straw for bedding was associated with a 13% reduction in stall discomfort index, while using wood shavings or sawdust was associated with a 20% reduction. Similarly, having a well-placed neck rail was associated with a 12% decrease in the discomfort index compared with stalls that had no neck rail or an improperly placed neck rail. There was a 2% decrease in discomfort index in stalls associated with each additional liter of milk produced by the cow (Table 3.7). Graphical and statistical assessment of normality and heteroskedasticity indicated the model had acceptable goodness-of-fit.

3.4.5 Exploration of scenarios for the impact of the intervention on discomfort index

Among the three scenarios of compliance explored, scenario three had a significant interaction between study group and visit number, as shown in Table 7 and demonstrated in Figure 4. This scenario had the effective intervention group based on compliance with at least 40% of recommendations versus a comparison group of control farms and 0% compliance farms (and removing those farms with compliance of 1-39%).

Based on this scenario grouping, stalls on effective intervention farms had a significantly lower discomfort index on the second visit than stalls on comparison farms on the second visit, while accounting for the baseline discomfort indices of the farms on the first visit. The other two scenarios did not lead to a significant interaction variable, although these scenarios did reveal a trend toward a lower discomfort index among stalls on effective intervention farms compared to comparison farms. The goodness-of-fit tests indicated the data had an acceptable fit for the models.

3.4.6 Factors associated with compliance of individual recommendations within farms

The following variables had significant ($p<0.25$) univariable associations with compliance; type of recommendation, number of recommendations given, person who received the recommendations, having attended a cow comfort training session in the last year, and current daily milk yield (Table 3.8). In the final mixed regression model, only the type of recommendation and number of recommendations given remained significant (Table 3.9). While controlling for random effects at the farm level, a minor recommendation had 13.6 times higher odds of compliance than a major recommendation. Similarly, having one fewer recommendation doubled the odds of compliance. The goodness-of-fit tests indicated the data had an acceptable fit for the model.

3.5 Discussion

The results of this study indicated that measures of cow comfort (i.e. stall hygiene and knee impact) can be enhanced by giving oral and written summaries of cow comfort recommendations that explain their implementation and effectiveness in the context of SDFs. There is no single measure that captures all facets of stall cow comfort; however, stall base hygiene and hardness capture a large part of what would appear to be important for stall cow comfort (Figure 3). The goals of the cow comfort recommendations were to provide improvements toward a stall that was soft, dry and clean.

The most common recommendations given to farmers (neck rail placement, bedding and improving the stall floor) show the important structural components of cow comfort that need improvement on most SDFs. While there were a number of farmers that knew the importance of having a neck rail and had one, most of them lacked the knowledge on positioning it properly, and thus, the neck rail had not been achieving its intended purpose on those farms. The frequency of recommendations given agrees with previous research (Kathambi et al 2019) in which neck rail placement and improving floor softness were also among recommendations given to most farmers. Most farmers complied with the neck rail placement and floor softness recommendations.

While the mean farm-level compliance score to the recommendations was measured at 49.0%, a 40% cutoff for compliance was noted as a natural break in the data, where intervention farms typically had compliance scores of less than or equal to 30% or more than 40% (Figure 2). Therefore, it made sense to use the “per protocol” principle for statistical analysis which involved restricting the intervention group to an effective intervention group of farms with a 40% or higher compliance score.

Compliance was highest with minor changes involving cleanliness, such as stall and alley cleanliness (Table 2). Recommendations that needed substantial construction (major changes) had lower compliance, such as adjusting the total length of the stall. This result was consistent with findings from a previous study (Kathambi et al., 2019). This result can be explained by the cost and labor implications of such major changes recommended to farms that had poorly constructed sheds. Most of these farms indicated that they had plans to erect better sheds in the future, but they were not yet financially ready for the undertaking.

When exploring different scenarios of compliance levels within groups in the interaction modeling, we were able to demonstrate a significant interaction between study group and visit number in the final multivariable model to confirm the impact of the intervention on the discomfort index, but only for scenario three. From this scenario three analysis, it would appear that farms with between 1 and 39% compliance were not well suited to be counted in the effective intervention or in the control group. We did not include other variables in this scenario three analysis because the random allocation of the trial should have balanced out selection biases, information biases and confounding biases for the groups. Furthermore, including specific recommendations in the model along with the intervention group would not be appropriate because the recommendations would be correlated with the intervention grouping.

There was a trend towards an improvement from the intervention when comparing the two groups and adjusting for the discomfort indices of the first visit for scenario one and two; however, the interaction variable lacked a significant coefficient in those models. The lack of a significant interaction in scenario one and two was partly due to our sample size not being quite adequate to demonstrate this effect. Also contributing to this non-significant interaction was the slightly lower discomfort index in the intervention group versus the control group at the baseline visit, and this interaction took into account this slightly different discomfort index starting point. Furthermore, there was large variability in the discomfort index, with some farms already having a low discomfort index (i.e. 2 or 3 out of 8); therefore, there was limited room for comfort improvement on those farms. Finally, contamination of the comparison group likely led to some improvement in the discomfort index on those farms as well.

Stall hygiene and knee impact were the only comfort parameters that were significantly different between the comparison and effective intervention groups during the second visit (Table 4), suggesting that they were the variables most impacted by the intervention recommendations. These two stall floor characteristics would be directly affected by stall characteristics such as dimensions, bedding and neck rail placement.

Bedding is a vital determinant of comfort since it affects softness, abrasiveness and dryness of the stall floor. Nearly half (Table 2) of farmers were given the recommendation to address stall hygiene, and over 80% either fully or partially implemented this recommendation, indicating good success of the intervention to addressing this concern. There was, however, some room for improvement in compliance to this recommendation. The most frequently used type of bedding was dry sawdust, while straw and rubber mattresses were rarely used in the region. Type of bedding was a significant model factor associated with the discomfort index using scenario one. The discomfort index was lower when the bedding used was sawdust or wood shavings, and for crop waste or straw, compared to the reference category (having no bedding) (Table 6). When sawdust or wood shavings were used, the discomfort index was numerically lower than crop waste or wood shavings, but not significantly so. A number of farmers indicated reluctance to use sawdust even when it was available, citing that it was associated with flea infestation to their cows. This reluctance could be traced back to dogs lying on the sawdust before it was used as cow bedding, leaving fleas and their eggs in the sawdust and shavings, which would infest cows later on, as reported by the farmers and observed by the first author.

A neck rail is another important component of cow comfort in a stall, as depicted by the model showing a well-positioned neck rail having a significant association with a reduced discomfort index. This result agrees with previous studies that have found improved lying time associated with proper placement of a neck rail (Kathambi et al., 2019). As the neck rail recommendation was given to most farmers, there is a large potential for improving comfort in the stalls on SDFs in Kenya. Nearly half (Table 2) of farmers given this recommendation either fully or partially implemented this recommendation, indicating some compliance success of the farmer-mediated intervention to addressing this concern, but with some room for improvement. Hopefully, the results of this study will be able to convince farmers of the importance for this cow comfort recommendation.

Current daily milk yield was also significantly associated with the discomfort index in the final model, although it is likely that this association is showing the higher milk production benefits of a low discomfort index rather than higher milk production being a predictor for low discomfort index. Influential observations of current daily milk yield were highlighted by leverage values larger than the calculated cut-point (0.0336). The most extreme leverage values were from cows that had very high milk production compared to the mean (10 litres). For example, the 2 cows that had the highest milk production (25 and 28 liters per day) had the highest leverage values (0.072 and 0.099, respectively). There was also a trend towards large leverage values for cows that had small daily milk yield values (e.g., below 5 litres).

Since the study was conducted on low-income smallholder dairy farms, our aim was to give recommendations that were easy to implement with limited resources and skills. Feedback from the intervention farmers showed that only a small proportion (13.5%) of farms encountered some type of challenges that hindered implementation of recommendations. Only 9.5% of farms incurred some cost associated with the recommendations. These results show that the recommendations were easy to implement with minimal resource requirements and had potential for bringing an improvement in comfort, and therefore welfare of the cows.

The prevalence of neck lesions was 5.3% lower in the comparison group than the effective intervention group during the first visit, and 4.0% lower during the second visit, suggesting that the differences in neck lesions by group over time were not related to the recommendations (Tables 3 and 4). This result was potentially influenced by cows being let out of the shed to graze more often after the first visit than before the first visit. This grazing change was because we conducted the study in the drier months of the year in the study area (August to November), during which, some farmers graze their cows on community pasture due to inadequate fodder to feed them in the shed. Neck lesions were not only caused by wrongly placed neck rails but also wrongly built wooden feeding troughs with an upper rail rubbing on the cows' necks. The neck rail and feed trough neck injuries have been observed in previous studies in Kenya (Aleri et al., 2011; Aleri et al., 2012) where animals grazing outside a zero-grazing shed were less likely to have neck lesions inflicted by the two structures.

The slight decrease in prevalence of lameness among farms between visit one and two can likely be attributed to the fact that we treated clinical cases, including lameness, during the first visit. Less lameness could also have been influenced by seasonality since the study was conducted during the drier months of the year with usually few foot rot cases associated with cows standing in a muddy or soggy pen.

Slight improvements in the control group in some comfort outcome variables, such as carpal and hock injuries, can be partly attributed to contamination between study groups. Previous studies (Kathambi et al., 2019) have found that farmers living in the same community are bound to have interactions with other farmers in different study groups. In this case, control farmers could have gotten some information on improving their farms from intervention farmers and could have attempted to apply them. Furthermore, the comparison group we used in the scenario one analysis included the allocated control group farms and some intervention farms that did not meet the threshold of implementing at least 40% of recommendations given. The intervention farms with poor compliance could have attempted to implement some of their recommendations, which could account for some of the improvements noted in the comparison group.

Future research to quantify the benefits of making cow comfort recommendations to smallholder dairy farmers in Kenya should consider a number of methodological refinements. Screening of herds prior to enrolment could select farms with high discomfort indices, which would provide more room for improvement in cow comfort, thereby making it easier to demonstrate positive improvements from implemented recommendations with the same number of farms. Also, the number of farms could be increased to improve the power to detect a significant difference between groups, although for the time frame for this project, additional time was not available to add more farms to the study. Finally, a block randomization could be used to balance the discomfort indices of farms between groups.

More research could also be done to explore the cost-benefit of cow comfort interventions which utilize locally available and inexpensive material, which should encourage intervention uptake. Research on incentivizing comfort interventions could also be conducted, such as providing rewards to farmers achieving the best improvements in cow comfort. Finally, we hope that a stall comfort scoring tool based on the characteristics of the stall floor, as utilized in this study, can be developed and validated in the future. The discomfort index used in this study could be adapted and adjusted as necessary for conventional use. Wide dissemination of research findings is warranted so that farmers can see the demonstrated impact of the cow comfort interventions.

The study was also limited in that assessment of partial and full compliance of minor and major recommendations was somewhat subjective and was based on the experience of the PI, with support from the research team. However, observations were made consistently by the same person on all farms, thereby reducing the bias brought about by the subjectivity of observations. Also, there was no blinding among the allocated intervention groups, which was logistically not possible.

This study was part of another study assessing mastitis prevention recommendations, which required cows to be not more than 2 months in milk. This requirement limited the sample size for this study since all cows fitting the inclusion criteria were included, providing good generalizability to SDFs in this region of Kenya, and other SDFs with similar management. With a larger sample size, some associations that only saw a trend towards being significant might become significant. Moreover, with the greater perceived direct benefit of implementing mastitis recommendations rather than comfort recommendations, especially on farms that had previous mastitis problems, there could have been reduced implementation of comfort recommendations, especially given the compliance model results showing there was lower compliance when there were more recommendations.

3.6 Conclusion

Our study determined that when the data were analyzed per protocol, the intervention significantly reduced the stall discomfort index on the SDFs. Among the intervention recommendations, improvements to stall floor hygiene and softness through farmer implementation of improved bedding management and neck rail placement on SDFs were shown to be the cow comfort recommendations that substantially improved the discomfort index.

Compliance with such recommendations is high when they are focused (few in number), inexpensive and can be done using locally available material. Since substantial discomfort indices were noted, there is need to educate SDFs in Kenya and other locations with similar management on the importance, methods, and benefits of having a comfortable stall.

3.7 Conflict of Interest Statement

The authors declare no conflicts of interest in all aspects of this study.

3.8 Acknowledgments.

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Table 3. 1: Comfort input parameters for assessment, and their definitions

Comfort Input parameter	How measured	Appropriate status/dimension
Roof	Visual assessment of roof	Complete without holes, extending at least 30 cm beyond the stall area
Surface water	Visual assessment of land grade	Flowing surface water able to drain away from the stall without wetting it.
Floor soft	Knee impact test (McFarland, 1991)	Free from hard objects (McFarland, 1991)
Floor dry	Knee wetness test (McFarland, 1991)	Free from wetness (McFarland, 1991)
Stall hygiene	Visual assessment (Reneau et al., 2005)	Free from accumulated dung, urine and pooled water
Floor flat	Stall floor visual inspection	Free from visual uneven elevations
Total width	Width of stall between adjacent walls or partitions. (Cook, 2009; Tucker et al., 2004)	Depends on size of cows (Cook, 2009; Tucker et al., 2004)
Total length	Length from rear curb to front of the stall where head is (Cook, 2009).	Depends on size of cow; should allow lunge space in front of brisket board (Cook, 2009)
Body length	Length between rear curb and neck rail and/or brisket board	Depends on size of cow; should allow cow to lie inside the stall comfortably

		without depositing manure or urine in the stall (Cook, 2009; Tucker et al., 2004)
Leg space	Space between lower dividing board and stall floor	Should be enough for cow to stretch legs below bottom divider while lying down.
Lunge space	Space in front of brisket board	Should be adequate for cow to lunge while rising
Neck rail	Measurement from stall base to underside of neck rail; and neck rail to back curb	Depends on size of cow; should be able to stand in stall fully without passing manure in the stall (Cook, 2009)
Brisket board	Measurement from stall base to back curb	Should be directly below the neck rail, maximum 25 cm above the floor (Tucker et al., 2004)
Alley way	Visual assessment of floor space where cow stands/walks while feeding	Floor can be walked on without getting boots dirty
Sharps	Visual assessment of sharp objects sticking out of the walls of the entire shed	There should be no sharps that could lead to cow injuries.

Table 3. 2: Comfort recommendations and compliance levels among 74 farms allocated to the intervention group

Comfort input parameter	**# of farms given recommendation**	**% of farms given recommendation**	**# of farms that fully complied**	**#of farms that partially complied**	**% of farms complied at least partially**
Fix roof	4	5.4	0	0	0
Fix surface water	21	28.4	3	4	33.3
Improve knee impact	26	35.1	12	6	69.2
Improve stall hygiene	36	48.6	16	14	83.3
Make floor flat	27	36.5	8	7	55.6
Improve stall width	18	24.3	5	1	33.3
Improve stall length	2	2.7	0	0	0
Improve leg space	13	17.6	5	5	76.9
Improve lunge space	9	12.2	6	0	66.7
Improve neck rail	61	82.4	21	9	49.2
Improve brisket board	28	37.8	2	4	21.4
Make alleyway clean	36	48.6	9	14	63.9
Fixing sharp edges	1	1.4	1	0	100

Table3. 3: Prevalence of comfort parameters on the baseline visit of 119 cows on 110 farms in Kenya

Comfort Parameter	Comparison Group [A]		Effective Intervention Group [A]		P-value	Overall	
	Prev (%)	**95% CI**	**Prev (%)**	**95%CI**		**Prev (%)**	**95% CI**
Udder hygiene	21.7	10.9 - 36.4	19.1	10.9 – 30.1	0.74	20.2	13.4 – 28.5
Leg hygiene	45.6	30.9 – 70.0	52.2	40.0 – 63.9	0.49	49.6	40.3 – 58.9
Stall hygiene	28.3	15.9 – 43.5	35.6	24.7 – 47.7	0.41	32.7	24.4 – 42.0
Knee impact	67.6	55.7 – 78.0	51.1	35.8 - 66.3	0.07	61.3	52.0 – 70.2
Neck injuries	15.2	6.34 – 28.9	20.5	12.0 – 31.6	0.47	18.5	12.0 – 26.6
Carpal injuries	4.35	0.53 – 14.8	2.74	0.3 – 9.55	0.64	3.36	0.92 – 8.38
Hock injuries	4.35	0.53 – 14.8	1.37	0.03 – 7.40	0.31	2.52	0.52 – 7.19
Lameness	13.0	4.94 – 26.3	2.74	0.33 – 9.54	0.03	6.72	2.95 – 12.8

[A] = Based on scenario one analysis: effective intervention group has farms with at least 40% compliance vs comparison group has farms with <40% compliance added to control

Table 3. 4: Prevalence of comfort parameters on the second visit of 119 cows on 110 farms in Kenya

Comfort Parameter	Comparison Group		Effective Intervention Group		P-value	Overall	
	Prev (%)	**95% CI**	**Prev (%)**	**95%CI**		**Prev (%)**	**95% CI**
Udder hygiene	21.6	12.9 – 32.7	13.3	3.7 – 24.1	0.26	17.6	11.3 – 25.7
Leg hygiene	39.2	28.0 – 51.2	28.9	16.4 – 44.3	0.37	36.1	27.5 – 45.4
Stall hygiene	40.0	24.7 – 52.4	17.8	8.0 – 32.1	**0.01**	31.3	22.9 – 40.6
Knee impact	58.1	46.0 – 69.5	24.4	12.9 – 39.5	**<0.01**	45.4	36.2 – 54.8
Neck injuries	2.70	0.33 – 9.42	6.67	1.40 – 18.3	0.30	4.20	1.37 – 9.53
Carpal injuries	1.35	0.03 – 7.3	0		0.43	0.84	0.02 – 4.59
Hock injuries	1.35	0.03 – 7.3	0		0.43	0.84	0.02 – 4.95
Lameness	5.41	1.87 – 10.6	2.22	0.05 – 11.8	0.40	4.20	1.38 – 9.53

Table3. 5: Univariable regression results for variables that had significant association with log of discomfort index (p=0.25) in 119 cows on 110 smallholder dairy farms in Kenya

Variable	Categories	Exponentiated Coefficient	95% CI	P-value
Visit number	1	Reference		
	2	0.92	0.85 - 0.99	0.05
Study group [B]	Comparison	Reference		0.01
	Intervention	0.88	0.81- 0.96	
Bedding type:	No bedding	Reference		<0.01[A]
	Straw/crop waste	0.82	0.75 - 0.90	<0.01
	Sawdust/wood shavings	0.73	0.67 - 0.80	<0.01
Neck rail	Absent/wrongly placed	Reference		
	Well placed	0.77	0.70 - 0.84	<0.01
Current daily milk yield	Milk in kg/day	0.98	0.97 - 0.99	<0.01

[A] = Global p-value for categorical variable.

[B] = Based on scenario one analysis: effective intervention group has farms with at least 40% compliance vs comparison group has farms with <40% compliance added to controls

Table 3.6: Final multivariable regression model of factors significantly associated with log of discomfort index (p=0.05) in 119 cows on 110 smallholder dairy farms in Kenya

Variable	Categories	Exponentiated coefficient	95 % C.I	P-value
Bedding type	No bedding	Reference		<0.01[A]
	Straw/crop waste	0.87	0.79 – 0.95	0.01
	Sawdust/wood shavings	0.80	0.73 – 0.87	<0.01
Neck rail	Absent/ wrongly placed	Reference		
	Placed appropriately	0.82	0.75 - 0.89	<0.01
Current milk yield	Milk in kg/day	0.98	0.97 - 0.99	<0.01

[A] = Global p-value for categorical variable

Table 3.7: Multivariable regression models with study group and visit number associations with log of discomfort index in 119 cows on 110 smallholder dairy farms in Kenya using 3 different scenarios of compliance utilized in the analysis

	Scenario one [A]		Scenario two [B]		Scenario three [C]	
Variable	**Exponentiated coefficients**	**P-value**	**Exponentiated coefficients**	**P-value**	**Exponentiated coefficients**	**P-value**
Study group	0.93	0.218	0.89	0.036	0.93	0.268
Visit number	0.96	0.401	0.97	0.648	0.97	0.648
Study group * Visit number Interaction	0.90	0.207	0.89	0.151	0.84	0.049

[A] = Effective intervention group has farms with at least 40% compliance vs comparison group has farms with <40% compliance added to controls

[B] = Effective intervention group has farms with at least 1 recommendation with at least partial compliance vs comparison group has farms with 0% compliance added to controls

[C] = Effective intervention group has farms with at least 40% compliance vs comparison group has farms with 0% compliance added to controls; farms with >0% but <40% compliance removed from dataset

Table 3. 8: Factors that were univariably associated with compliance of 239 individual recommendations given to 70 smallholder dairy farms in Kenya

Factor	Levels	Odds Ratio	95% CI	P-value
Type of recommendation	Major	Reference		
	Minor	16.9	5 – 100	<0.001
Number of recommendations given	-	2.0	1.3- 3.3	0.003
Principal farmer	Male	Reference		0.03 [A]
	Female	2.5	0.5 - 13.9	0.29
	Other	0.3	0.04 - 1.6	0.14
Attended cow comfort training in last year	No	Reference		
	Yes	0.3	0.09 - 1.4	0.14
Current daily milk yield	Milk in kg/day	1.1	0.9 - 1.4	0.24

[A] = Global p-value of categorical variable

Table 3. 9: Final mixed multivariable regression model of factors associated with compliance of 239 individual recommendations given to 70 smallholder dairy farms in Kenya

Factors	Levels	Odds ratio	95% CI	P-value
Type of recommendation	Major	Reference	3.4 – 53.7	
	Minor	13.6		<0.001
Number of recommendations given	-	2.0	1.3 – 3.3	0.008

Figure 3. 1: Flow chart for selection and allocation of the 114 smallholder dairy farms recruited into the study

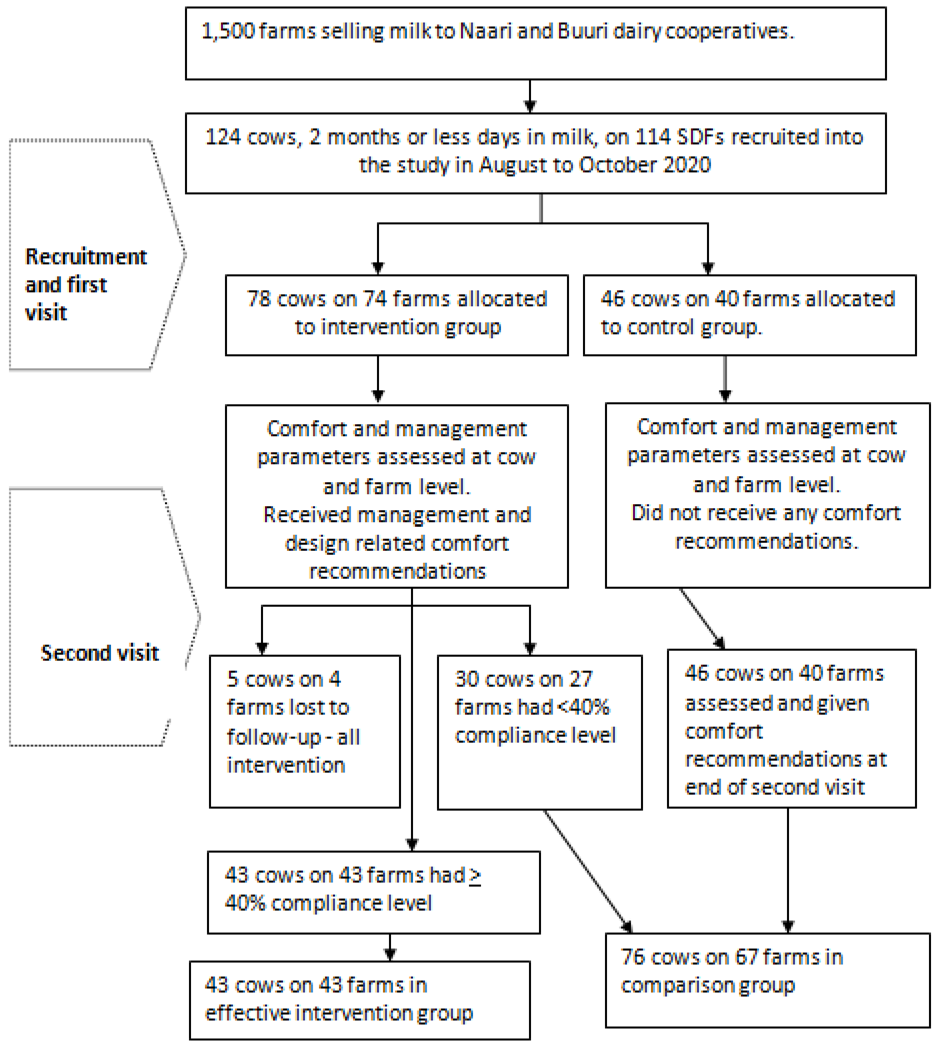


Figure 3. 2: Histogram depicting compliance scores among 60 allocated intervention farms on their second visit

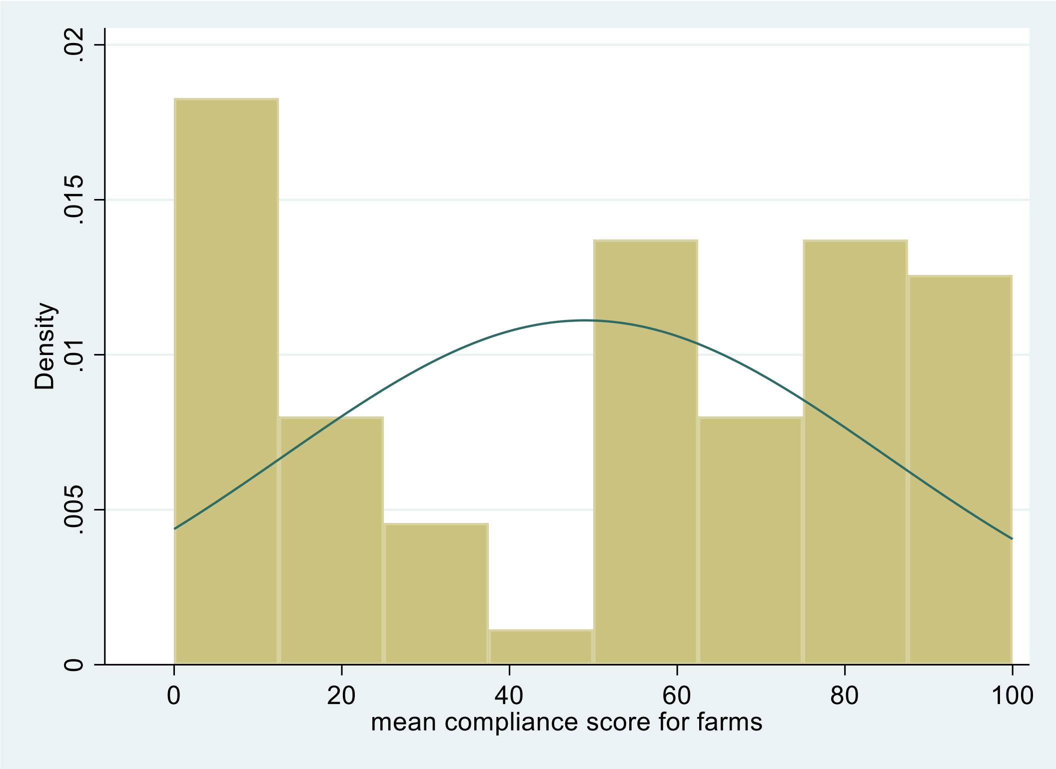


Figure 3. 3: Causal diagram to explore associations of various stall characteristics to knee impact and stall hygiene

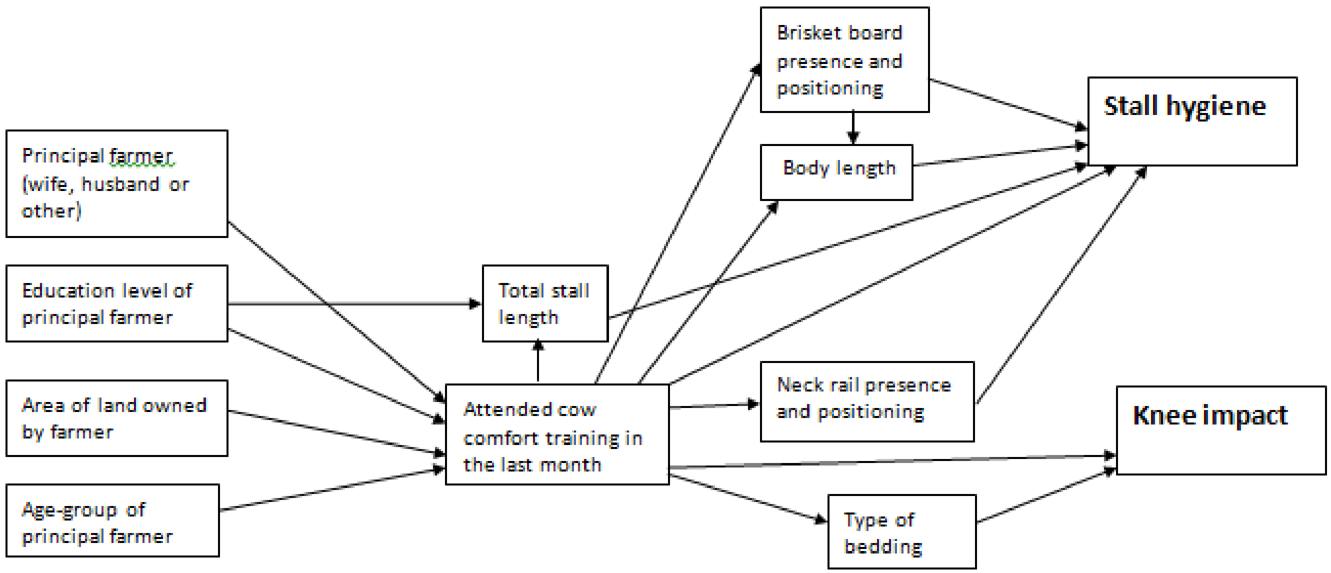
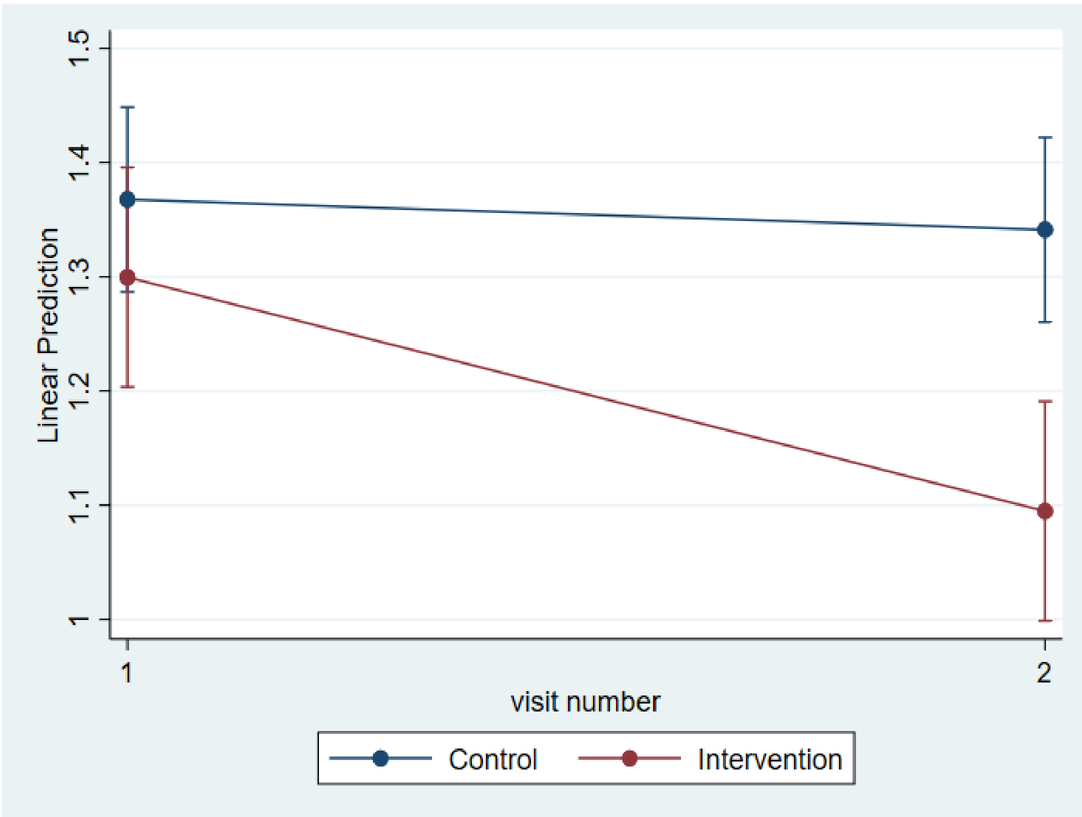


Figure 3. 4: Predictive margins plot of the interaction between study group and visit number, demonstrating their association with log of discomfort index in 103 cows on 97 smallholder dairy farms in Kenya



Chapter 4: Investigating compliance with cow comfort and mastitis control recommendations on mastitis outcomes in smallholder dairy farms in central Kenya

4.1 Abstract

A randomized controlled trial was conducted in Kenya in 2020 with the objectives being to assess compliance of smallholder dairy farmers with farm-specific mastitis and cow comfort recommendations, to determine factors associated with compliance, and to determine the impact of these recommendations in reducing cases of subclinical mastitis.

A total of 124 cows were recruited into the study and randomly allocated into intervention and control groups during the first visit in August 2020. Existing mastitis control protocols and cow comfort were assessed in both intervention and control farms. A California Mastitis Test (CMT) was done on all quarters and scored from 0 (negative) to 3 (strong positive). All quarters with a CMT score of 2 and 3 were treated for mastitis. Intervention farms received farm-specific mastitis and cow comfort recommendations at the end of the first visit. On the second visit, CMT was repeated on all quarters on all cows with CMT 2 were treated for mastitis. Compliance with recommendations was assessed and given a percentage score. Control farms were then given recommendations. Regression models were utilized to assess associations with compliance and improvement in CMT scores between visits.

Intervention farms received an average of 3.3 and 3.9 recommendations pertaining to mastitis control and cow comfort, respectively. Farms complied with an average of 2.8 and 2.1 mastitis and cow comfort recommendations, respectively, leading to an overall average compliance score of 63.2% for all recommendations. On the first visit, CMT scores 1 and 2 were significantly higher in the intervention than control cows. On the second visit, there was

a significantly lower proportion of quarters with CMT scores 1 and 2 in the intervention group on the second visit than the first visit and no quarters with CMT score of 3. The principal farmer, type of recommendation and number of recommendations given were significantly associated with compliance to the recommendations. The principal farmer and their age group were significantly associated with quarter CMT scores improving between the first and second visits.

In conclusion, farm-specific mastitis and cow comfort recommendations can significantly reduce the number of cases of subclinical mastitis in SDFs and should thus be included in farmers' training. Farmers should be given a small number of recommendations at a time to enhance compliance.

4.2 Introduction

Mastitis is the most important infectious disease contributing to economic losses in dairy production around the world (Awale et al., 2012b) causing an estimated loss of $35 billion (Modi et al., 2012). It accounts for the majority of use of antimicrobials on dairy farms (Redding et al., 2014). Bovine mastitis is the inflammation of the mammary tissue in cows which can manifest physically with gross spoilage of milk, swelling, reddening and warmth in the affected mammary tissue, in which case it is called clinical mastitis. It can also fail to manifest physically in the cow, in which case it is called subclinical mastitis (SCM). In both cases however, there is an influx of immune cells in the affected quarter as a response to the infection, and desquamation of the epithelial lining of mammary alveoli (Bradley & Green, 2009; Harmon, 1994). California Mastitis Test (CMT) is a rapid cow-side test for detecting

SCM. Somatic cell count (SCC) is another ideal technique of detecting SCM. While it is easy to diagnose clinical mastitis physically, subclinical mastitis presents the challenge of early detection and thus can remain in a dairy herd, causing losses while still unestablished. Subclinical mastitis manifests with a positive alcohol test or the milk curdles after boiling. This leads to milk rejection at the collecting point or processor, and it cannot be used for domestic consumption.

Bovine mastitis may be classified epidemiologically as either environmental or contagious (Cervinkova et al., 2013). Environmental mastitis is caused primarily by pathogens whose main reservoir is the cow's environment. These are associated with an unhygienic environment. Commonly isolated organisms include most bacteria of the family Enterobactericiae, *Staphylococcus* species other than *S. aureus* and *Streptococcus* other than *Streptococcus agalactiae* (Smith et al., 1985). Contagious mastitis is caused by pathogens whose primary reservoir is the infected cow's mammary tissue and are spread to other cows primarily during the milking process. *Staphylococcus aureus* and *Streptococcus agalactiae* are the most diagnosed organisms (Radostits et al., 2007; Riekerink et al., 2006).

Staphylococcus and *Streptococcus* species is associated with majority of bovine mastitis cases all over the world (Islam, Rahman, et al., 2012; Sharma et al., 2011; Shitandi & Sternesjö, 2004). Contagious mastitis is of paramount concern to dairy producers for two main reasons. First, the organism invades the mammary tissue and replicates there, being subsequently shed intermittently in milk, posing the risk of spreading to other cows in the same herd during the milking process. Second, some strains of *Staphylococcus aureus*, are able to produce a biofilm and/or microabscesses which prevent antibiotics and the host's immune factors from accessing the bacterial cells, rendering treatment using antibiotics less effective (Karzis et al., 2018).

In developing countries, smallholder dairy farmers (SDF) account for the majority of milk produced (Bonilla et al., 2017; Peeler & Omore, 1997). Most of these farms have less than five cows which they depend on as their source of livelihood (Randolph et al., 2007). Dairy cows in these farms are housed in simple structures usually made from timber and less often from concrete blocks. Average daily milk yield ranges from 8-15 liters per cow per day (Bundi et al., 2014; Gitau et al., 1994; Kathambi et al., 2018) with a big proportion of animals producing less than 10 liters, but with potential for improvement in milk production. As such, these farms can benefit a lot from improved mastitis control.

Numerous approaches have been employed in the control of bovine mastitis with varying degrees of effectiveness. In particular, antimicrobials have been used extensively in treatment and prevention of mastitis. Mastitis has, however, persisted as a key factor limiting dairy productivity, and thus the need for continued effort in improving its control. Antimicrobial resistance has progressively persisted as a setback to efficiency of controlling mastitis using antimicrobials. This has necessitated exploration of alternative ways of controlling mastitis on dairy farms such as integrating routine management with health management (Abdi et al., 2018; Barkema et al., 2006b, 2006a).

AMR has spurred a global initiative towards antimicrobial stewardship which among other things aims at finding ways of reducing the need to use antimicrobials in production animals in managing infectious diseases. On dairy farms, these efforts are anchored on routine management and husbandry practices that promote optimal welfare and comfort. Studies have demonstrated that optimal comfort and welfare have a direct impact in promoting good health in animals (Barkema et al., 2006b; Karzis et al., 2018) thus reducing the need for antimicrobial use (AMU) and promoting sustainable productivity. Comfort parameters in a dairy housing structure include a well-drained and clean alley space, a soft and dry sleeping stall with appropriate dimensions, an appropriate feed bank and an appropriate and well drained milking parlor (Cook et al., 2005b; Tucker, Weary, Rushen, et al., 2004). Dairy housing unit built with these considerations provides a suitable environment with minimal stress and a low chance of the animal getting injured within the pen thereby aiding in maintaining good general health.

Consequent to the challenges around the use of medicines, there has been a growing need to explore approaches and practices around husbandry and management that can help in the control of mastitis, as well as to evaluate the effectiveness of such approaches. A previous study (Kathambi, VanLeeuwen, Gitau, & Revie, 2019) demonstrated improved comfort from implementing a set of comfort recommendations, but failed to demonstrate any benefit on mastitis control. This study was aimed at exploring benefits of comfort and mastitis control recommendations on control of bovine mastitis in smallholder dairy farms.

4.3 Materials and Methods

4.3.1 Study area and population

The study was conducted in Buuri Sub-county of Meru County in Central Kenya. Meru is situated approximately 290km north of Kenya's capital, Nairobi. It is at an altitude of about

1930m above sea level, and lies at latitudes 0.14 º North and longitudes 37.57º East. The area receives an average annual rainfall of 1800mm. Most residents in this region are small scale farmers who practice mixed farming.

We purposively recruited all farms that had cows two months or less in milk and were shipping milk to Naari and Buuri dairy farmers' cooperatives. In total, there are close to 1500 farmers attached to these two cooperatives. For farms to qualify for the study, they also had to have a zero-grazing unit, and own not more than five adult dairy cows (those in milk and dry ones) at the time of the study. Lastly, farmers had to be willing to participate in the study. Only those cows that were 2 months or less in milk were included for mastitis testing and sampling.

4.3.2 Ethical approval

The study was approved by the Research Ethics Board and the Animal Care Committee of the University of Prince Edward Island as well as The Sir James Dunn Animal Welfare Centre, who partly funded it. Approval was also obtained from Naari and Buuri dairy cooperative societies and Farmers Helping Farmers, a partnering non-governmental organization that works with the two dairy cooperatives. Written consent was sought from individual farmers during the first visit.

4.3.3 Data collection

Data were collected from farms during two visits done two months apart. The study was a randomized controlled trial (RCT) where block randomization was done and all farms that met inclusion criteria in the blocks were recruited. On the first visit, farm- and cow- specific data were collected through a questionnaire and from direct observation and measurement where appropriate. Farmers were asked about their current mastitis control protocols, about production of their cows, feeding, and the occurrence of mastitis on their farms in the last year. Milk production records were verified where they were available. They were also asked who the principal farmer was, designated as the person involved in the daily management of the dairy cows. Milking hygiene protocols were recorded and observed if the visit coincided with milking time. Milking hygiene practices of interest included use of individual reusable udder towels for every cow and whether each towel was washed and dried after every milking, whether milkers washed their hands using detergent before milking, and whether mastitic quarters and cows were milked last.

Cow parameters were measured directly or observed from the cow. Body condition was scored from 1 (very thin) to 5 (obese) (Wildman et al., 1982). Height was measured using a tape measure at the level of the withers, with a level placed horizontally on the withers for precise measurement. Weight was approximated using a heart girth band passed around the chest just behind the fore limbs. All quarters from all cows were tested for subclinical mastitis using California Mastitis Test (CMT) by placing approximately 5ml of milk from each individual quarter into a corresponding well of a CMT paddle, adding an equal amount of CMT solution and swirling the mixture for 10 seconds. The mixture was poured out of the mixture was gently poured from the paddle and results were read and scored based on color and consistency the as 0 (negative), 1 (weak positive), 2 (distinct positive) and 3 (strong positive) (National Mastitis Council, 2004). Milk samples were asceptically obtained from quarters with CMT 2 or 3 and then treated using a commercially available cephapirin sodium intramammary tube (Cefalak, Boehringer Ingelheim, Burlington, ON, Canada).

Cow comfort parameters were evaluated in the stall based on the cow's weight. The presence and placement of a neck rail and brisket board, stall total length, body length and stall width were measured as stipulated in previous studies (Cook, 2009a; Tucker, Weary, & Fraser, 2004). Stall floor hardness was assessed by means of a knee test (McFarland, 1991) and scored on a scale of 1 (soft) to 3 (hard). Stall hygiene and cow hygiene of the upper hind legs, and udder were observed and scored 1 (clean) to 5 (dirty) (Reneau et al., 2005). Cows were observed for injuries on the neck, carpi and hocks, and the injuries scored on a scale of 0 (absent) to 3 (severe injuries) (Dairy farmers of Canada, 2021). At the end of the first visit, farm-specific mastitis control and cow comfort recommendations were given to intervention farms, as described under the intervention section.

4.3.4 Intervention

Based on the conditions and management on each farm, farmers were given up to 5 mastitis control recommendations from a list of 6 that were previously identified as ideal for SDFs and required minimal resources to comply with (Natonal Mastitis Counsil, 1994). The recommendations were either tagged as major, where the mastitis control practice related to that recommendation was not existent, or minor, where it was existent but required improvement. Similarly, farmers were given up to 8 cow comfort recommendations from a list of 10 that were previously identified as ideal for SDFs and required minimal resources to comply with (Cook, 2009a; Tucker, Weary, Rushen, et al., 2004). The recommendations were either tagged as major, where the cow comfort practice related to that recommendation was absent, or minor, where it was present but needed improvement. An intensity mark was given for each recommendation as follows; 0 for a recommendation not given, 1 for a minor recommendation and 2 for a major recommendation.

On the second visit, farm- and cow-specific characteristics were re-evaluated and compliance with recommendations was recorded. CMT was again conducted on all quarters, and quarters with CMT of 2 or 3 were sampled and treated with Cefalak®. Control farms were given their farm-specific mastitis control recommendations so as to eventually match their counterparts in intervention group, as a thank you to their participation in the study.

4.3.5 Second visit assessment of compliance

On the second visit, compliance to the comfort and mastitis recommendations was assessed and marked as 0 where there was no change, 1 where there was an attempt to implement the recommendation, but it was not implemented completely and/or correctly, and 2 where the recommendation was fully and correctly implemented.

For the maximum possible compliance score for each recommendation, the recommendation intensity mark (0, 1 or 2) was multiplied by the highest possible mark for compliance (2), and all of the maximum compliance scores for each recommendation were added together for a total maximum possible compliance score for each farm. To determine the compliance score for each recommendation, the intensity mark was multiplied by the actual achieved compliance mark for each recommendation, and all of the compliance scores for each recommendation were added together for a total compliance score for on each farm. A percentage compliance score was calculated for each farm by dividing the total compliance score by the total maximum possible compliance score and multiplying by 100. Recommendations not given did not count in the percentage compliance score because their intensity mark was zero.

4.3.6 Laboratory analyses

Laboratory analyses were conducted at the Department of Clinical Studies (CSD) laboratory and the Department of Public Health Pharmacology and Toxicology (PHPT) of the University of Nairobi's Faculty of Veterinary Medicine. Milk samples which were stored in frozen state were thawed on the bench at room temperature for two hours. A loop-full from each sample was streaked on blood and MacConkey agars (Himedia®) and incubated aerobically at 37 degrees Celsius for 24 hours (National Mastitis Council (U.S.), 2004). Colony characteristics were recorded. A Gram stain was conducted and a catalyse test was used to differentiate *Staphylococcus* from *Streptococcus* species, while a coagulase test was conducted to differentiate between *S. aureus* and coagulase-negative *Staphylococcus* (Quinn et al., 1994).

For antimicrobial sensitivity testing, a 0.5 McFarland's standard was prepared using pure fresh colonies from each isolate from each respective sample (McFarland, 1991). Antimicrobial testing was done using a modified Kirby Bauer disc diffusion method (Tankeshwar, 2013). Antibiotics tested for the sensitivity testing included tetracycline, penicillin G, ampiclox, cephalexin, kanamycin, streptomycin and gentamicin. The inoculated agar with the multidiscs were incubated at 37 degrees Celsius aerobically for 24 hours. Sensitivity to the various antibiotics was reported by the diameter of the zone of inhibition of growth, measured in millimeters, according to Clinical Laboratory Standards Institute standards (Wayne, 2018).

4.3.7 Data handling and analysis

Data were coded and entered into Microsoft Excel 2010 (Microsoft lnc, Sacramento, California, USA) where they were cross-checked and imported into Stata 16.1 (Stat Corp LLC, College Station, Texas, USA) for statistical analysis. For data analysis, CMT scores were dichotomized as negative (0) and positive (scores 1 and above). Prevalence of subclinical mastitis was then determined as a percentage of all cows in the study that had a CMT score of one and above. Changes in CMT scores between groups and between visits within groups were assessed using a 2-sample proportion test.

Two logistic regression models were run, one to determine factors associated with compliance with mastitis control recommendations, and the second to determine the impact of the intervention on CMT score of 0 or 1 at the baseline. For the latter model, quarters that had baseline CMT score more than 1 were removed since these were treated with antibiotics at the first visit. Quarters that had CMT 1 on the first visit and were 1 or higher on the second visit were categorized as positive quarters on visit 2, together with quarters that had CMT 0 on the first visit and CMT 1 or higher on the second visit since the intention of the intervention was to improve mastitis control conditions for both contagious and environmental mastitis on the second visit.

An “effective intervention group” variable comprising of farmers that had at least 40% compliance for both mastitis control and comfort recommendations was forced into the model since it was the primary factor of interest in the model. Allocated intervention farms that did not have a total compliance score of at least 40% were combined with the control farms to create a "comparison group" of farms, based on results from a related study showing a natural division in compliance percentages to cow comfort recommendations (Kariuki et al., 2023). This method of data analysis utilizes the "per protocol" strategy, whereas analyzing based on allocated intervention and control groups would follow the "intent to treat" strategy.

For the modeling, variables were initially assessed for univariable association at $P<0.25$ significance level. Those variables meeting the univariable criteria were eligible for the multivariable modeling using manual backward selection (Dohoo et al., 2010), ($P<0.05$). Confounding variables were assessed by checking for at least a 20% change in coefficients when any variable that was $P<0.25$ and not in the final models were re-entered into the final models. Two-way interaction was also assessed among variables in the final model. Hosmer-Lemeshow Chi-squared test was used to assess the fit of the models.

4.4 Results

4.4.1 Study population

The study initially included 124 cows on 114 farms having at most 5 milking cows at the time of the study. Cows in the study were mainly Friesians (59.7%) while other breeds such as Ayrshires (18.6%), Guernsey (7.3%), Jerseys (1.6%) and their various crosses (11.3%) were less common. A small proportion of farms reared indigenous cows (1.7%). On the first visit, the body condition of these cows ranged from 1.0 to 3.5, with 65.3% having a body condition score of 2.5 and above. They weighed on average, 350 kg (±69.5 s.d.) ranging from 230 – 698 kg, and had an average daily milk yield of 10.7 (±4.28) litres, ranging from 1.5 to 28 litres.

Among the 110 farms that remained in the study, on 27.3% of farms (n=30), the principal farmer was male family member, and on 40.9% of farms (n=45) the principal farmer was female family member. On 30.9% of farms (n=34), either a hired person or a relative was the principal farmer, playing the main role in the daily operations of the farm. On 1 farm it was not clear who was the principal farmer. Of these 110 principal farmers, 60 were less than 50 years of age, 43 were over 50, while 8 did not disclose their age.

4.4.2 Description of recommendation and compliance results

Based on the milking hygiene protocol present on each farm, intervention farms received a minimum of 1 and a maximum of 5 (average 3.3) milking hygiene protocol recommendations. On average, farmers complied with 2.8 recommendations. Table 1 shows the individual milking hygiene protocol recommendations, their frequency of being recommended, and the farmer's compliance to those recommendations. Iodine teat dip use and washing hands before and between cow milking were the most common recommendations, and at least partial compliance to these two recommendations was over 90%. The mean compliance percentage for milking hygiene recommendations was 77% with a minimum of 3.3% and a maximum of 100%.

Based on the status of cow comfort indicators observed on each farm, intervention farms received a minimum of 0 and a maximum of 7 (average 3.9) recommendations pertaining to cow comfort. Table 4.2 shows the frequency of cow comfort recommendations given to intervention farms and their compliance levels. Improving neck rail placement and improving stall floor hygiene were given to most farmers while creating adequate leg room and improving stall floor hygiene had the highest compliance levels. Increasing total length of the stall was given to two farms which did not attempt to make the recommendation, thus attaining 0% compliance score. The mean compliance percentage for comfort recommendations was 49.8%. Overall, the mean compliance for both comfort and milking hygiene recommendations was 63.2%**,** ranging from 1.7% to 100%.

4.4.3 Comparing baseline to follow-up CMT results

At the beginning of the study, the percentages of quarters with each respective CMT score above zero were significantly higher in the intervention group than control group (Table 4.3), based on the 2-sample proportion test. Therefore, there was significantly more subclinical mastitis among the intervention quarters than in control quarters at the beginning of the study.

Table 4.4 shows that there was a larger increase in quarters with CMT score 0 and a larger decrease in quarters with CMT score 1 or higher in the intervention group versus the control group when comparing CMT results in the first and second visits. The CMT results were quite similar between visits in the control group. The proportion of quarters with CMT 0 increased from 72.4% to 89.4% from visit 1 to visit 2 in the intervention group.

The visit two CMT 1 quarters are a combination of first visit quarters with CMT score 1 that remained CMT score 1 and first visit quarters with CMT score > 1 or <1 that became CMT score 1 on visit two. From Table 4.4, there is evidence of benefit to being an intervention quarter due to the significant reduction in number of CMT score 1 quarters during the second visit in the intervention group compared to no change at all in the comparison group.

4.4.4 Logistic regression model results

Table 4.5 shows the final multivariable logistic regression model of three factors associated with compliance with mastitis control recommendations. On a farm where the principal farmer was female, compliance with recommendations was close to twice as likely compared to where a male family member was the principal farmer. Where it was neither of the two (it was a relative or an employee), compliance was almost half as likely. A minor recommendation had 23 times higher odds of compliance than a major recommendation. Similarly, farms that received 2 or 3 recommendations had a third of the odds of compliance compared to those that received only one recommendation. Those farms receiving more than three recommendations had reduced odds of compliance by almost 10-fold compared to when 1 recommendation was received (Table 4.5).

Table 4.6 shows the final multivariable logistic regression model of two factors associated with CMT scores remaining/becoming zero on the second visit, from quarters that were 0 or 1 on the first visit. Where the wife was the principal farmer, odds of a quarter that had CMT score 0 or 1 on the first visit remaining/becoming zero CMT score on the second visit were diminished to 0.18. Where the principal farmer was someone other than the husband or the wife, the odds were also diminished to 0.15, compared to the husband being the principal farmer.

When effective intervention was defined to include only farms that implemented at least 40% of each set of recommendations (Table 4.7), being an effective intervention group quarter rather than a comparison group quarter proved to confer strong benefit by reducing the proportion of quarters with CMT 1 between first and second visits ($p<0.001$).

4.4.5 Bacterial Culture

On visits one and two, there were 46 and 17 quarters that were CMT score 2 or 3, respectively, requiring sampling for bacterial culture (Tables 4.3 and 4.4). Of these samples, those that had growth after culture were relatively few, at 15 of 46 and 6 of 17, producing proportions of 34.1% and 35.3%, respectively.

Of the 17 quarters with CMT score 2 or 3 on visit 2, 5 (31%) were infected on the 1st visit and remained infected. All of the 63 quarters with CMT >1 on the first and second visit were treated with an intra-mammary antibiotic infusion. From Table 4.8, being an intervention farm did not seem to confer protection against new infections or retained infections since the proportions were not significantly different versus the effective comparison group.

All culture-positive quarters on the second visit had no growth on the first visit, although some of these culture-positive quarters had CMT scores above 1 on both visits (Table 4.8). Of the culture-positive quarters from both visits, there were only two quarters from the same cow. From the first visit quarters only, there was one cow with all four quarters being culture-positive, one cow with three quarters being culture-positive, and another cow with two culture-positive quarters. Only *Staphylococcus aureus* organisms were isolated from the samples that had growth. The culture results were grouped as persistent infections and new infections.

4.4.6 Antibiotic resistance

The isolated organisms (n=21) were all resistant to penicillin, and all isolates showed at least intermediate sensitivity to cephalexin, a 1^{st} generation cephalosporin. All isolates exhibited at least intermediate susceptibility to gentamicin (an aminoglycosides) and tetracycline (Table 4.9).

Based on first and second visit culture results, there was no substantial change in antimicrobial susceptibility. The following drugs were tested but were not reported due to lack of appropriate interpretive criteria for cattle or humans; ampicillin, ampiclox, kanamysin and streptomycin.

4.5 Discussion

This is the first study assessing compliance with recommendations on both milking hygiene and cow comfort in SDFs in low-income countries. The study also determined the effectiveness of recommendations in improving mastitis control in SDFs in Kenya providing the basis on which these recommendations can be utilized to improve mastitis control.

Block randomization was preferred to help minimize possibility of contamination between study groups. Farmers at the edges of the blocks still had some chance of interaction which would have led to such contamination. This can limit effectiveness of the intervention resulting from farmers in the control group attempting to apply some of the recommendations given to their intervention group neighbors and resulting in failure to demonstrate significant differences between study groups. Fortunately, the CMT results in the control group were largely unchanged from the first to second visit, demonstrating that the potential for contamination bias was likely minimal.

At the beginning of the study, the higher prevalence of mastitis in the intervention group would present an ideal chance to demonstrate improvement on the second visit from implementing recommendations on the first visit. This improvement was observed on the second visit with intervention farms having lower prevalence of mastitis. However, this difference between groups at the baseline was contrary to equal prevalence within groups as we would have expected with randomized allocation of farms. However, a larger sample size would likely have led to similar prevalence of mastitis between groups.

Quarters that had CMT 2 or 3 on the first visit were treated using an antibiotic intra-mammary preparation (Cefalak ®) since the farmers relied on their cows for milk production and daily income as a means of livelihood. Quarters with CMT score 1 did not receive the treatment and thus, following them up on the second visit, along with CMT 0 quarters, would give an unbiased assessment of the impact of compliance with the recommendations.

Improving the conditions in the cows' environment such as hygiene (Barkema et al., 2006a; Klaas & Zadoks, 2018) is known to be effective in controlling environmental mastitis while employing appropriate milking hygiene protocols is known to be effective in controlling contagious mastitis. Regarding new infections, the cow comfort interventions should lead to fewer environmental mastitis infections but not necessarily lead to fewer contagious mastitis infections, while the milking hygiene intervention should have led to fewer environmental and contagious mastitis infections. Therefore, compared to the intervention group, there is a higher likelihood of cross-infection between quarters of an already CMT-positive cow with a contagious pathogen such as *S. aureus* in the control/comparison group. Similarly, there is a higher likelihood of infection spread between CMT-positive cows with contagious pathogens such as *S. aureus* in the control/comparison versus intervention group. The results from follow-up CMT assessment may be susceptible to some bias arising from failure to blind the observer who did the assessment on the second visit. However, given the researchers had ample experience conducting CMTs, and they conducted the CMTs prior to assessing compliance, this concern is not likely to be biasing the results.

The results from CMT testing provide indications of inflammation in the udder, which often is a function of a current intra-mammary infection, but the inflammation could be from other causes, such as trauma or a previous intra-mammary infection that is resolved. Culture results would provide additional information to understand the benefits of the interventions. Unfortunately, the low number of samples with culture results meant limited information on the etiology of the CMT-positive quarters.

The overall compliance score agrees with a previous study (Kathambi et al., 2019) that found 66% compliance, which was the basis of recruiting more intervention farms in this study. The decision to include only those farms that had at least 40% compliance in intervention related analysis was guided by trends in compliance data. For cow comfort recommendations compliance scores in Chapter 3, the trend was bimodal with majority of farmers having either more than 40% or less than 40% with very few being around 40%

(Figure 3.2). From the average compliance results of comfort and milking hygiene recommendations, the trend was unimodeal but that the proportions at 40% or higher compliance are all similar (0.013 or 0.014), and is less frequent to be < 40% (<0.007) (Figure 4.1).

4.5.1 Regression models

From the regression models, compliance with recommendations was significantly associated with the gender of the principal farmer, type of recommendation (whether major or minor) and the number of recommendations. Other studies have also shown that giving fewer recommendations was associated with higher compliance levels (Kathambi, VanLeeuwen, Gitau, & Revie, 2019)

Women play a central role in most activities in smallholder dairy farms and provide most of the labour (Chavangi & Hansen, 1983; Kimaro et al., 2013; Maarse et al., 1995). This observation can explain why compliance was significantly higher when women were the principal farmers on intervention farms. This is directly related to why there was a significant difference in improvement of CMT scores on farms where women were the principal farmer.

4.5.2 Milk culture and antimicrobial sensitivity

Based on previous experience with mastitis cultures in low-producing cows managed in Kenyan smallholder dairy farms (Bundi et al., 2014), a quarter was to be sampled if CMT score was 2 or 3. Not only did this lead to fewer samples to be cultured, it may have had a predisposition to bacteria that produce a high inflammatory response, such as *S. aureus*. However, *S. aureus* is also known to be shed intermittently in milk samples, due to formation of micro-abscesses, potentially leading to no growth on a single milk sample (Sears et al., 1990). *S. aureus* is a contagious mastitis agent that is associated with chronic mastitis cases and has suboptimal cure rates by lactation intra-mammary infusion treatment (Sharma et al., 2011; Sol et al., 1997).

The low frequency of isolates can be partly attributed to issues around sample storage. The samples were stored in a freezer from day 1 of sampling to the last day of the second round of visits. Due to unreliable power supply in the research area, we put in the freezer some icepacks to try to maintain the samples in a frozen state during power outages since repeated freeze-thaw cycles can reduce the survival of certain bacteria, particularly environmental mastitis pathogens, (Schukken et al., 1989). Unfortunately, there were some power outages during the study period, likely leading to some repeated freeze/thaw cycles, despite our efforts to minimize this problem.

For isolates recovered, results from this study agree with previous studies in Kenya which found *S. aureus* to be the predominant organism isolated (Gitau et al., 1994; Mureithi & Njuguna, 2016; Shitandi & Sternesjö, 2004). This result was contrary to findings from a different study in the same region (Mbindyo et al., 2020) that found *Streptococcus agalactiae* as the predominant agent with *S. aureus* coming third after coagulase negative *Staphylococcus*.

Despite tetracycline being widely used to treat cattle through systemic administration in Kenya, it still exhibited high activity against the isolated mastitis-causing organisms. This observation is likely because tetracycline is not often used in intra-mammary preparations in Kenya, and as such, common mastogenic bacteria have not had long time exposure to teracyccline. Tetracycline is known to have limited availability in mammary tissue following systemic administration (Lents et al., 2002). Alternatively, most organisms susceptible to other antimicrobials commonly used in intramammry antimicrobial infusions (e.g. gentamicin) may have been eliminated by those antimicrobials over time, since they are commonly used in intra-mammary preparations in Kenya, leaving tetracycline susceptible organisms which are tending to respond to the tetracycline in the test. Cephalexin was tested as the only first-generation cephalosporin available in place of cefapirin, which was used to treat quarters with CMT 2 and 3. The zone diameters used were for human interpretive criteria so they should be interpreted with caution.

Kanamycin and cephalexin are present in Terrexine (Bimeda, Naiobi, Kenya) a commonly used intra-mammary preparation in Kenya. Its wide use in treating mastitis is likely to account for the high-level resistance observed for cephalexin and moderate resistance for Kanamycin. Gentamast® (Bremer Pharma GMBH, Warbug Germany) is also a common intra-mammary preparation which contains gentamicin, for which most isolates were sensitive. Due to several technical challenges in the laboratory, all antimicrobial resistance test results should be interpreted with caution.

4.5.3 Opportunities for future research

The intention of the study was to demonstrate that compliance with recommendations improved mastitis results in intervention farms and not in control/comparison farms. It would be beneficial if the person doing the follow-up assessment was blinded to eliminate the possibility of bias. The person observing the CMT results could bias results towards achievement of the improvement in the intervention group.

The time span for this project was limited to 4 months because it was to be completed for a Masters degree time frame; the follow-up period could not be extended beyond the stipulated timelines. The results of this project would have yielded better evidence if the follow-up period would have been longer, allowing more time for farmers to comply with the recommendations. More than one follow-up visit may have also instigated better compliance. More farms in the study would also be warranted to enable a higher power to detect differences between intervention and control farms. These factors can be incorporated in future research for better validity of results.

Sampling quarters only if they were CMT 2 or 3 limited the number of samples taken for culture. Sampling quarters with CMT score 1 would not only improve the sample size of the culture results for statistical analyses but would also inform us more about which bacteria were being cultured from these positive quarters. Comparing isolation rates from fresh cultures and from frozen samples would also be informative to guide future research about storage of milk samples for culture since we suspect that using frozen samples had a role in the low isolation rate and diversity of isolates observed in this study.

4.6 Conclusions and Recommendations

The results of the trial demonstrated a trend to better udder health as measured by CMTs from the combination of recommendation interventions addressing milking hygiene protocols and cow comfort. Subclinical mastitis remains prevalent in SDFs, and the associated losses continue to be an impediment to higher milk production. There is a need to better understand factors that influence its occurrence and how they can be applied to control mastitis. Since the compliance with mastitis control recommendations was high, farmers were enthusiastic to implement recommendations to prevent associated losses from treatment and production reductions. Since SDFs have limited understanding on the process and benefits of improvements to cow comfort, methods of improving the low compliance with cow comfort recommendations are needed. Cow comfort topics should be incorporated in farmers' training to enhance their understanding of the subject.

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4.8 Conflict of Interest Statement

The authors declare they had no conflicting interests in the study.

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Table 4. 1: Mastitis control recommendations given to 74 intervention SDFs in Kenya in 2020 and their respective compliance levels

Mastitis control parameter	# of farms given recommendation	% of farms given recommendation	# of farms that fully complied	# of farms that partially complied	% of farms complied at least partially
Iodine teat dip use	74	100	64	10	100
Wash hands before and between milking multiple cows	70	94.6	53	12	92.9
Udder of each cow washed using a	38	51.4	19	2	63.2

different towel					
Milking parlor hygiene	44	59.5	5	16	47.8
Clean towel to clean and dry udder	13	17.6	4	4	61.5
Mastitic cow/teat milked last	1	1.4	1	0	100

Table 4. 2: Cow comfort recommendations given given to 74 intervention SDFs in Kenya in 2020 and their compliance levels.

Comfort parameter	# of farms given recommendation	% of farms given recommendation	# of farms that fully complied	#of farms that partially complied	% of farms complied at least partially
Roof water	4	5.4	0	0	0
Surface water	21	28.4	3	4	33.3
Floor soft/dry	59	79.8	23	22	76.3
Floor flat	27	36.5	8	7	55.6
Total width of sleeping stall	18	24.3	5	1	33.3
Total length	2	2.7	0	0	0
Leg space	13	17.6	5	5	76.9
Lunge space	9	12.2	6	0	66.7
Fix Neck rail	61	82.4	21	9	49.2
Fix Brisket board	28	37.8	2	4	21.4
Alley clean	36	48.6	9	14	63.9
Sharps fix	1	1.4	1	0	100

Table 4. 3: Quarter-level California Mastitis Test (CMT) results from first visits of 114 farms in August and September 2020 in Kenya, by allocated control and intervention groups.

CMT score	Allocated Control Farms		Allocated Intervention Farms		P-value for difference
	n	%	n	%	
Score 0	164	89.1	226	72.4	<0.001
Score 1	12	6.52	47	15.1	0.004
Score 2	7	3.80	28	8.97	0.03
Score 3	1	0.54	10	3.21	0.06
Non-functional quarter	0	0	1	0.32	
Totals	**184**	**100**	**312**	**100**	

Table 4. 4: Quarter-level California Mastitis Test (CMT) results from second visits of 110 farms in October and November 2020 in Kenya

CMT score	Effective Control Farms		Effective Intervention Farms		P value
	n	%	n	%	
Score 0	260	87.8	161	89.4	0.59
Score 1	26	8.78	11	6.11	0.29
Score 2	10	3.38	7	3.89	0.77
Score 3	0	_	0	0	
Non-functional Quarter	0	0	1	0.56	
Totals	**296**	**100**	**180**	**100**	

Table 4. 5: Factors associated with compliance with mastitis control recommendations given to 110 SDFs in Kenya in 2020

Variable	Levels	Odds Ratio	P-value	95% CI
Principal farmer	Man	Reference	[A]0.039	
	Woman	1.9	0.234	0.66 - 5.6
	Other	0.6	0.264	0.22 – 1.5
Type of recommendation	Major	Reference		
	Minor	23.5	<0.001	5.4 – 102.5
Number of recommendations	1	Reference	[A]0.049	
	2 or 3	0.3	0.240	0.03 – 2.4
	>3	0.1	0.059	0.01 – 1.1

[A] – Global p-value

Table4. 6: Variables associated with CMT score 0 having a higher CMT score or CMT score 1 quarters either remaining as 1 or having a higher score on the second visit

Variable	Levels	Odds ratio	P-value	95% CI
Principal farmer	Man	Reference	0.003[A]	
	Woman	0.18	0.004	0.02 - 0.57
	Other (employee/relative)	0.15	0.005	0.04 – 0.56
Age group of principal farmer	<50 years	Reference		
	>=50 years	0.17	0.004	0.05 – 0.57
>=40% compliance for mastitis and comfort recommendations	<40	Reference		
	>40	0.65	0.432	0.22 – 1.88

[A] – global p-value for categorical variable.

Table4. 7: Changes in quarter level California Mastitis Test score 1 between first and second visits on farms in Kenya, August to November 2020, by intervention group, where intervention farms complied with at least 40% of each set of recommendations

Study group	First visit			Second visit			P-value -difference within groups between visits
	n	N[A]	%	n	N	%	
Effective							
Intervention Farms	34	211	16.1	11	211	5.21	<0.001
Effective							
Comparison Farms	25	264	9.47	26	264	9.85	0.88
Total	59	475	12.4	37	475	7.79	
P-value for difference within visits between groups		0.03			0.06		

[A]Total number of quarters from cows in respective study group

Table 4. 8: Quarter-level culture growth results for milk samples with California Mastitis Test above 1, from 1st and 2nd visits of farms in Kenya, August-November 2020, by intervention group, where intervention farms complied with at least one of each

Study Group [A]	**1st visit**			**2nd visit**					
				Remained infected			**New infections**		
	n	**N**	**%**	**n**	**N** [A]	**%**	**n**	**N** [A]	**%**
Effective Intervention Farms	9	211	4.27	0	9	0	3	202	1.49
Effective Control Farms	6	264	2.27	0	6	0	3	258	1.16
Total	**15**	**475**	**3.15**	**0**	**15**	**0**	**6**	**460**	**1.30**
P-value for differences between groups within visit			**0.22**			**n/a**			**0.76**

[A] – Total number of isolates from respective study groups.

Table 4. 9: Quarter level sensitivity results for positive cultures on the first and second visits of farms in Kenya, August-November 2020, measured by zone of inhibition in mm

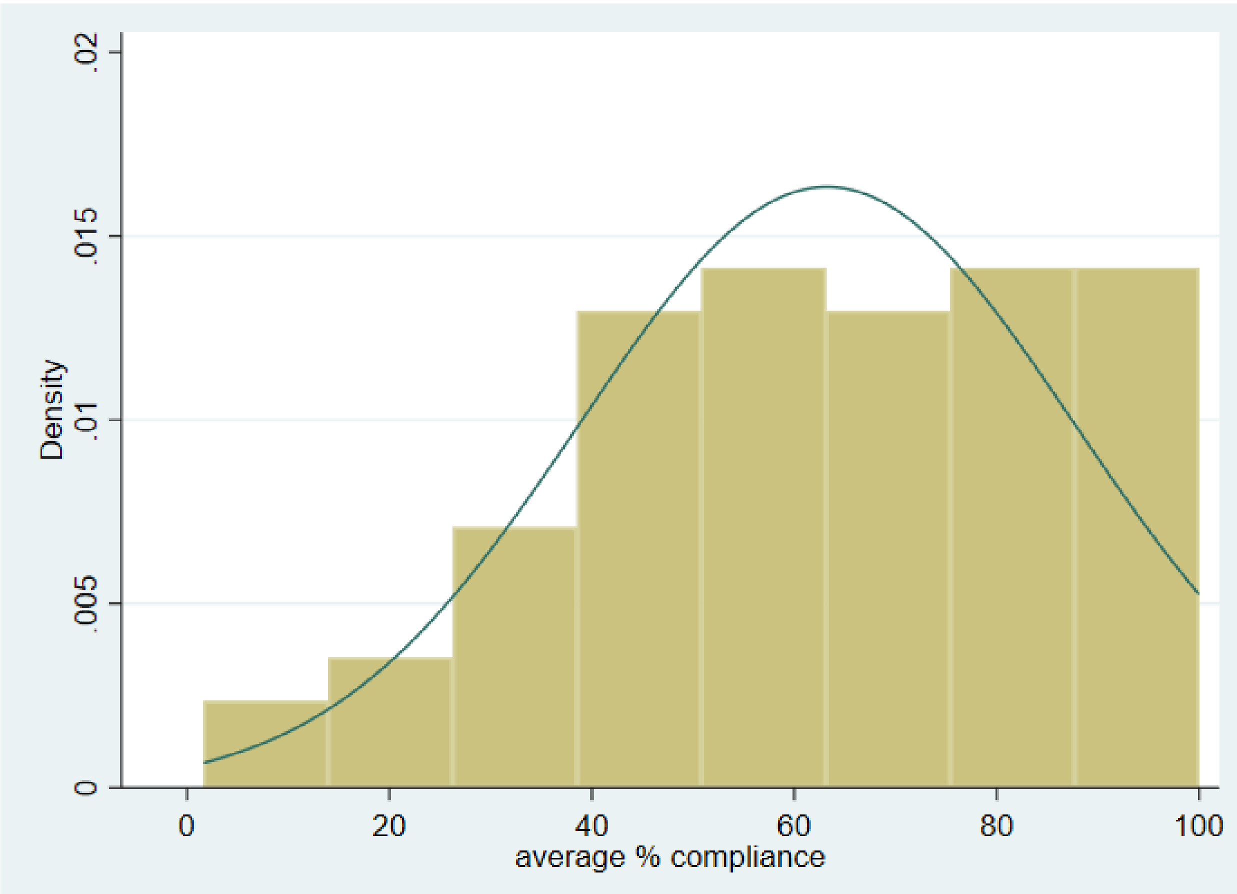
Drug	Susceptible			Intermediately susceptible			Resistant		
	N	zone [A]	%	N	zone [A]	%	N	zone [A]	%
Penicillin[A]	0	(>29)	0	0	(na)	0	21	(=<28)	100
Gentamicin[A]	21	(=>15)	100	0	(13-14)	0	0	(=<12)	0
Cefalexin[B]	17	(>21)	81.0	4	(18-20)	19.0	19	(<0)	0
Tetracycline[A]	12	(=>19)	57.1	9	(15-18)	42.8	0	(<15)	0

A – Human interpretive criteria were used since there was no ideal criterion for cattle

B – Interpretive criteria for cattle mastitis based on ceftiofur was used since it was the only cephalosporin with interpretive criteria.

Figures

Figure 4. 1: Histogram of percentage compliance scores for both cow comfort and mastitis control recommendations given to 70 intervention farms in Meru, Kenya.



Chapter 5: A summary chapter of the thesis methods, results, conclusions and recommendations

5.1 Introduction

Knowledge about control of subclinical mastitis is limited among smallholder dairy farms (SDFs) in Kenya (Awale et al., 2012a; Gitau et al., 1994). Inadequate resources to implement mastitis control protocols on SDFs also limit the efforts to control the disease. In developed countries, there is a relatively good understanding of mastitis control measures due to ample research efforts. Unfortunately, many of the scientific advances gained in developed countries are not directly applicable to SDFs in developing countries due to, among other factors, inadequate resources of SDFs. Since mastitis contributes to a substantial proportion of losses due to disease in SDFs (Awale et al., 2012b; Mungube et al., 2005; Romero et al., 2018; Schepers & Dijkhuizen, 1991), there is need to incorporate mastitis control measures that are directly applicable to SDFs and are inexpensive to implement. Understanding how recommendations to SDFs for better cow comfort and mastitis control can impact cow comfort and mastitis levels on SDFs would be very helpful scientific knowledge.

The overall objective of the thesis was to explore the impact of integrated mastitis and cow comfort controls on SDFs. Specific thesis objectives were: 1) to assess the status of cow comfort and subclinical mastitis control protocols in SDFs and determine factors associated with subclinical mastitis; 2) to determine the level of compliance with cow comfort recommendations and their impacts on cow comfort on SDFs; and 3) to determine the level of compliance with cow comfort and mastitis control recommendations and their impacts on subclinical mastitis on SDFs.

Objectives 2 and 3 were addressed by the same RCT, with objective 2 focused on the cow comfort recommendations and impacts, while objective 3 examined the combined effect of cow comfort and mastitis control recommendations on a different outcome subclinical mastitis. The first objective is a summary of the baseline findings from the same RCT addressing objectives 2 and 3. The objectives were separated in this manner to allow for chapters of a reasonable length, given the volume of data generated. Each objective/chapter is described in turn.

The thesis research was conducted in Meru County, Central Kenya, with an aim of reducing mastitis through solutions that can be easily applied to SDFs. The research was conducted among members of two dairy cooperatives; Naari and Buuri Dairy Cooperative Societies. The cooperatives consist of 1,500 farmers who were actively shipping milk at the time of the research. From these member farms, participating farmers were selected based on the following criteria: 1) farmers who were actively shipping milk to either of the two dairy cooperatives; 2) farmers who had at least one fresh cow not more than 3 months in milk; and 3) farmers who had not more than 5 adult dairy cows, either milking or dry.

5.2 Cross-sectional study of cow comfort and management factors associated with subclinical mastitis in smallholder dairy farms in Kenya

In this study, 124 cows from 114 SDFs were recruited. Cows were observed for hygiene on the upper hind legs and udder (Reneau et al., 2005), and body condition score was assessed following guidelines by Wildman et al., (1982). Sleeping stalls were observed for floor hardness and wetness (McFarland, 1991) and stall dimensions were measured and assessed for adequacy based on weight and industry standards

(Cook, 2009b; Tucker, Weary, Rushen, et al., 2004). Lameness was observed while cows walked within their cow shed and assigned a severity score (Sprecher et al., 1997). Quarter level mastitis was assessed using California Mastitis Test (CMT) (National MastitisCounsil, 1994). In addition to descriptive statistical analyses, multivariable logistic regression models determined animal- and farm-level factors associated with cow-level subclinical mastitis based on positive CMT results on at least one quarter.

Most farmers reared exotic breeds, including Friesians (59.7%) Ayrshires (18.6%), Guernsey (7.3%), Jerseys (1.6%) and their various crosses (11.3%). A small proportion of farms reared indigenous cows (1.7%). The body condition of the recruited cows ranged from 1.0 to 3.5, with 65.3% having a body condition score of 2.5 and above. They weighed, on average, 350 kg (±69.5 s.d.), ranging from 230 – 698 kg, and had an average daily milk yield of 10.7 (±4.28) litres, ranging from 1.5 to 28 litres.

Cow-level and quarter-level SCM prevalence found in this study (43.2% and 21.9%, respectively) was lower than reported in previous studies in Kenya (Mureithi & Njuguna, 2016). Those authors found cow-level prevalence of 64% and quarter-level prevalence of 55.8%. Cow-level prevalences of 56% and 65% were found in two other districts in Kenya (Bundi et al., 2014). The lower prevalence reported in the current study could be seasonal variation, or it could be attributed to the farmer assistance and education program offered by livestock stakeholders, such as Farmers Helping Farmers, a non-governmental organization, working with farmers in the region.

Existing mastitis control and milking hygiene practices were assessed on each farm. All farmers were milking their cows by hand. Most of the farmers were giving fresh feed after milking (87.0%), washing hands between milking different cows (80.7%), milking mastitic teats last (91.7%), and milking mastitic cows last (65.9%), where applicable. However, other mastitis control measures were infrequently employed, such as using different towels to wash each cow udder (49.5%), using dry cow therapy (10.1%), using disinfectant teat dip (10.1%), and stripping the first milk to assess physical changes suggestive of mastitis (21.1%).

The study cows were all reared in zero-grazing units, and 87.2% of farms had freestalls with partitions between stalls. Among the 95 farms with separate stalls for individual cows, 38 (40%) had a neck rail, of which 18 were well-positioned and 20 were inappropriately positioned. Of these 95 farms with separate stalls, 11 (11.6%) had a brisket board, of which 3 and 8 farms had poorly and well-positioned brisket boards, respectively.

A majority of the stalls (94.1%) had an earthen floor, while 5 (4.2%) had concrete floors and 2 (1.7%) had a wooden stall floor. Two-thirds of the stalls (67.8%) had bedding in the stall. Regarding bedding types, 31.4% had crop waste, 31.4% had wood shavings or saw dust, 2.5% had additional loose soil, and 0.8% was straw. The remaining 40 cows had no bedding, although rubber mats were used for two of these cows.

On a scale of 1 (very clean) to 5 (very dirty), 16 (13.6%), 63 (53.4%), 31 (26.3%), and 7 (5.9%) stalls were scored as 1, 2, 3, and 4, respectively, with 1 stall scoring 5. When recategorized on a dichotomous scale, 66.9% of stalls were scored as clean (scores 1 and 2) on the day of the visit while 33.1% of stalls were categorized as having a dirty floor surface (score 3, 4 or 5). In terms of hygiene, 49% of all cows had dirty legs, while 20% of udders were dirty. Alleyways were largely categorized as clean (82%).

Our final multivariable logistic regression model found that cows that were lying down in stalls with poor hygiene were associated with more subclinical mastitis on CMT results. This was an important association which has not been often reported in SDF. None of the mastitis control efforts was significant in the final model of factors associated with subclinical mastitis, which may be a function of the limited variability among farms of the mastitis control protocols.

There was an interaction in the final model between alleyway hygiene and current daily milk yield; for cows in a shed with a clean alleyway, probability of having SCM decreased with increasing milk yield, while those cows housed in a shed with a dirty alleyway had increased probability of having SCM with increasing milk yield. This result demonstrates the importance of a clean-living environment for dairy cows, not just the stall.

There was a negative correlation observed between alleyway and stall hygiene levels, which could be explained by farmers not cleaning the alleyway as often as the stall, or not cleaning the stall as often as the alleyway. This negative correlation could also suggest that some cows were not using their stalls and preferred lying down in the alleyway due to a stall with inappropriate dimensions for the various rails used for the stall (e.g. short stall length). Cows that don't lie down in the often are less likely to pass manure or urine in the stall, so it remains clean. Cows lying down on a dirty alleyway have been found to be more likely to have higher incidence of mastitis (Kathambi, VanLeeuwen, Gitau, & Revie, 2019; Kerro & Tareke, 2003; Lakew et al., 2009; Mungube et al., 2005) compared to those lying down in a clean stall. This result also greatly underpins the important interplay between proper stall design and management and various mastitis control protocols in the effective management of udder infections in these farms. We expected to find significantly fewer cases of SCM in cows that were treated with dry cow therapy and/or teat dip disinfectant. However, the farms that utilized either of these products were few (10.1%), and so we could not detect significant associations to these factors. The low use of these management tools can be attributed to both low knowledge and resources among farmers in the study. A majority of the farmers that were not using these products reported that they were not aware that such products existed. Being cross-sectional in nature, results from the study are not reliable to make a causal inference about the outcome from the predictors, as there is no element of temporality between the two. The model predictor variables were assessed at the same time as the outcome, making it impossible to confirm that they existed prior to the outcome. The study was also prone to recall bias, with farms that had their cows treated for mastitis previously likely being more conversant with routine mastitis control protocols that were advised by the veterinarian that treated their cows. Since a majority of the farmers had low-to-moderate knowledge levels in mastitis control, such farmers with a previous encounter with mastitis could still be practicing most of the protocols advised by the veterinarian, compared to farmers that had not had a cow treated for mastitis on their farms.

It would be of benefit if a cohort study or trial could be conducted to validate the outcomes of this study and establish a causal relationship between the factors incriminated in the occurrence of SCM.

5.3: Investigating compliance with and impacts of cow comfort recommendations in smallholder dairy farms in Meru region, Kenya

This study was a randomized controlled trial (RCT) conducted in Naari area of Meru County, Kenya. Objectives of the study were: 1) to assess compliance with comfort recommendations in SDFs, 2) to investigate the impact of compliance with these recommendations on the comfort status of cows on these farms.

To achieve the objectives, 124 cows on 114 farms were recruited to the study. These farms were divided into an intervention group with 78 cows on 74 farms and a control group with 46 cows on 40 farms. The intervention group was allocated more farms intentionally since compliance was expected to be around 66%, guided by findings from a previous study (Kathambi et al., 2019). In the course of the study, five cows from 4 for farms, all from the intervention group, were lost to follow-up (due to sale of cows that were recruited), leaving 73 cows on 70 farms in the intervention and 46 cows on 40 farms in the control group.

The study included two farm visits two months apart. On the first visit, farm- and cow-level data were collected, and farm-specific comfort improvement recommendations were given to intervention farms (see below). On the second visit, comfort parameters were reassessed and recorded. Compliance with the recommendations was assessed and given a percentage score. Control farms were also given recommendations on this second visit as a token of appreciation for participating in the study.

Intervention farms received specific comfort recommendations for their farm circumstances, and these recommendations were drawn from a list of 12 recommendations that were previously identified as suitable for SDFs (Kathambi et al, 2019; Cook 2009; Tucker et al., 2004). Recommendations were recorded as either major or minor, depending on how much construction was needed to bring each of them to a desirable level. Major recommendations carried more weight than minor recommendations at the time of assessment for compliance. Recommendations were given to the farmers in their local Kimeru dialect orally and in written format.

For this trial, a stall discomfort index was developed arithmetically by combining the scores of stall hygiene (1-5) and stall hardness (1-3). These parameters were considered inherently important determinants of stall comfort, with an ideal stall floor being clean, soft and dry. In addition to descriptive statistical analyses, univariable and multivariable linear regression modeling was utilized to determine variables associated with: 1) the overall compliance percentage for each farm; and 2) the discomfort index for each stall. We also determined if intervention group and visit number were associated with discomfort index.

Of all the comfort recommendations given, fixing a neck rail was given to the most farms (82.4%). One-third to one-half of farms were given the following recommendations: improve the stall base softness, improve the stall base hygiene, and improve the alleyway hygiene. Improving the brisket board placement was also a very common recommendation since only 12% of stalls with partitions had brisket boards. Creating ample leg space was recommended on 18% of farms.

The mean compliance for all cow comfort recommendations was 49.0%, lower than that reported in a previous study (Kathambi et al., 2019). For individual recommendations, improving stall hygiene (83.3%), creating ample leg space (76.9%) and making the stall floor soft (76.3%) had the highest compliance levels. Recommendations that involved considerable construction, such as changing the roof and changing the total length of a stall, had zero compliance, emphasizing the need to give inexpensive recommendations to SDFs.

Among the 70 allocated intervention farms that had a second visit, 43 farms having 43 study cows had a compliance score of at least 40% (becoming the effective intervention group) while 27 farms had lower compliance scores. The latter group of farms was grouped together with the 40 allocated control group farms to form a comparison group comprising of 76 cows on 67 farms. The mean compliance score among farms with 40% or more compliance was 75.1%.

The overall discomfort index ranged between 2 and 8, with a median of 3 and a mean of 3.7. The mean discomfort index on visit 1 was 3.9 and 4.0 for the allocated intervention and control groups, respectively (p=0.303). However, on visit 2, the discomfort index means were 3.3 and 4.0 for the effective intervention group and comparison group, respectively (p=0.003).There was a significant difference in both stall hygiene (p=0.012) and knee impact scores (p<0.001) between groups for the second visit, with the intervention group being better than the comparison group. The two parameters were also found to have significant differences in scores between the first and second visit within the intervention group.

The following variables had significant association (p<0.25) with discomfort index in univariate linear regression analyses; visit number, study group, type of bedding, neck rail positioning, and current daily milk yield. In the final multivariable linear regression model, bedding type, neck rail positioning, and current daily milk yield were significantly associated with discomfort index (p=0.05). The study by Kathambi et al., (2019) also found neck rail to be an important determinant of stall comfort. While controlling for effects of other variables in the model, using crop waste and straw for bedding was associated with a 13% reduction in discomfort index, while using wood shavings or sawdust was associated with a 20% reduction. Similarly, having a well-placed neck rail was associated with a 12% decrease in the discomfort index compared with stalls that had no neck rail. There was a 2% reduction in discomfort index for every additional liter of milk produced per day; cows that were producing more milk were using a stall in which discomfort index was lower.

The following variables had significant univariable linear regression associations ($p<0.25$) with compliance; receiving major recommendations, number of recommendations given, person who received recommendations, having attended a cow comfort training in the last year and current daily milk yield. In the final multivariable mixed linear regression model, while controlling for variance at the farm level, a minor recommendation had 13.6 times higher odds of compliance than a major recommendation. Similarly, having one less recommendation doubled the odds of compliance.

These findings were similar to findings from Kathambi et al., (2019) which found higher compliance when there were fewer recommendations given, and when recommendations were minor, not major. Being the recommendation given to most farmers, neck rail placement depicts the large potential that exists in improving comfort in the stall by placing a neck rail appropriately. Minor recommendations required fewer resources to implement, underpinning the need to give recommendations that can be implemented using minimal and preferentially locally available resources.

In conclusion, using appropriate bedding and placing a proper neck rail in the stall can significantly improve comfort status of sleeping stalls in SDFs. Giving farm-specific comfort recommendations can improve the status of comfort in SDFs, especially if there is good compliance. Giving farmers fewer recommendations at a time can enhance compliance and thus effectiveness of the comfort improvement. Giving minor recommendations was identified to be an important determinant of compliance. These insights should be incorporated in routine farmer training and education programs.

5.4 Investigating compliance with cow comfort and mastitis control recommendations and their impacts on subclinical mastitis in smallholder dairy farms in central Kenya

A randomized controlled trial was conducted to determine compliance with a combination of mastitis control and comfort improvement recommendations and their effectiveness on reducing subclinical mastitis in SDFs in Kenya. The study was conducted between August and December 2020 in which 124 cows on 114 farms were recruited. These were allocated into an intervention group (n=78 cows in 74 farms) and a control group (n=46 cows on 40 farms).

Farm-level and cow-level variables were observed on the first visit. Farmers were asked about their existing mastitis control protocols, and where possible, these were observed and recorded. Sleeping stalls were observed for hygiene, floor hardness and wetness, and stall dimensions for respective cows using the stalls. Hygiene was also observed on cow upper hind quarters. CMT was conducted on all quarters and milk samples were collected from quarters that had CMT score >1 for culture and isolation. Details of these procedures can be found earlier in this chapter.

Intervention farms were given a maximum of 5 farm-specific mastitis control recommendations from a list of 6 previously identified to be suitable for SDFs and a maximum of 7 comfort recommendations from a list of 12 previously identified as suitable for SDFs (Cook, 2009b; Kathambi, VanLeeuwen, Gitau, & Revie, 2019; National MastitisCounsil, 1994; Tucker, Weary, Rushen, et al., 2004). Recommendations were marked as either minor (1) or major (2) based on the effort required to implement the recommendation, or absent (0) for farms where they were not given. In addition to descriptive statistical analyses, multivariable logistic regression models were fit to explore associations with compliance for the given recommendations, as well as associations with resolution of CMT 1 quarters from first to second visit.

Among the 110 farms that remained in the study, on 27.3% of farms (n=30), a man was the principal farmer, on 40.9% of farms (n=45) a woman was the principal farmer, while on 30.9% of farms (n=34), either a hired person or a relative was the principal farmer, playing the main role in the daily operations of the farm. On one farm it was not clear who was the principal farmer. Of these 110 principal farmers, 60 were less than 50 years of age, 43 were over 50, and 7 did not disclose their age and it was not possible to correctly place them in either category.

Intervention farms received a minimum of 1 and a maximum of 5 (average 3.3) milking hygiene protocol recommendations. On average, farmers complied with 2.8 recommendations. Iodine teat dip use and washing hands before and between cow milking were the most common recommendations, and at least partial compliance to these two recommendations was over 90%. The mean farm-level compliance percentage for milking hygiene recommendations was 77% with a minimum of 3.3% and a maximum of 100%. Percentage compliance among farms that had at least 40% compliance was 81.0%

Based on the status of cow comfort indicators observed on each farm, intervention farms also received a minimum of 0 and a maximum of 7 (average 3.9) recommendations pertaining to cow comfort. Improving neck rail placement and improving stall floor hygiene were given to most farmers while creating adequate leg room and improving stall floor hygiene had the highest compliance levels. The overall mean compliance percentage for comfort recommendations was 49.8%. The mean compliance percentage among farms that had at least 40% compliance for cow comfort recommendations was 75.1%.

Overall, the mean compliance for both comfort and milking hygiene recommendations was 63.2%**,** ranging from 1.7% to 100%.

Based on the 2-sample proportion test, there was a significantly higher prevalence of subclinical mastitis among intervention farms than control farms on the first visit. This was contrary to an even distribution that would be expected from random allocation of farms to study groups. However, it formed a basis for demonstrating improvement in the intervention farm at the end of the study. The CMT results were similar between visits in the control group. In the intervention group, there was substantial increase in proportion of quarters with CMT 0 between visits and a decrease in proportion with CMT score 1 and above. For example, in the intervention group, proportion of quarters with CMT 0 increased from 72.4% to 89.4% from visit 1 to visit 2 while in the control group, it only increased from 87.8% to 89.1%. Although there were numerical differences in number of quarters with respective CMT scores, there was no significant difference between control and intervention group quarters, likely because of the higher proportion of quarters with CMT score greater than 1 in the intervention group than the control group at visit one.

Quarters that had CMT scores >1 were treated with intra-mammary antibiotic infusion during both visits. For this reason, comparing CMT 0 and 1 quarters at visit one with the second visit would be a fair comparison without bias resulting from treatment of other quarters. On the second visit, the proportion of quarters with CMT score 1 was significantly lower in the intervention group compared to the comparison group.

The following variables had significant association with compliance for the mastitis control and cow comfort recommendations in the final multivariable regression model: principal farmer, type of recommendation (major or minor) and number of recommendations. On a farm where a woman was the principal farmer, compliance with recommendations was close to twice as likely (OR=1.9=) compared to where a man was the principal farmer. Where it was neither the wife nor the husband (a relative or an employee), compliance was almost half as likely (OR=0.6). A minor recommendation had 23.5 times higher odds of compliance than a major recommendation. Similarly, farms that received 2 or 3 recommendations had a third of the odds of compliance compared to those that received only one recommendation (OR=0.3). Those farms receiving more than three recommendations had reduced odds of compliance by almost 10-fold compared to when only 1 recommendation was received. Women play a key role in running SDFs, as depicted from the higher odds of compliance from women-run farms. This has been documented from past studies where women were found to play a key role and provide most of the labour on SDFs (Chavangi N A & Hansen A, 1983; Kimaro et al., 2013; Maarse L W et al., 1995).

In the logistic regression analysis to explore associations with resolution of CMT 0 and 1 quarters, variables that had significant univariable association ($p<0.25$) were principal farmer, age group of principal farmer and farms with >40% compliance for both mastitis and comfort recommendations. In the final multivariable regression model, where the wife was the principal farmer, odds of a quarter that had CMT score 0 or 1 on the first visit remaining/becoming CMT score 1 on the second visit were diminished to 0.18. Where the principal farmer was someone other than the husband or the wife, the odds were also diminished to 0.15, compared to the husband being the principal farmer.

From these findings, we stipulated that older farmers, more than 50 years of age, were associated with fewer quarters having a worse score on the second visit since most of them were retired from other activities and were concentrating on farming as their main economic activity. Most young farmers were in other income-generating activities, including formal employment, and thus not being involved fully in the daily running of activities on their farms. We forced the intervention variable into the model since it was a variable of interest in this model, though it did not attain statistical significance.

On visits one and two, there were 46 and 17 quarters that were CMT score 2 or 3, respectively, requiring sampling for bacterial culture (Tables 3 and 4). Of these samples, those that had growth after culture were relatively few, at 15 of 46 and 6 of 17, producing growth proportions of 34.1% and 35.3% on first and second visit, respectively.

Of the 17 quarters with CMT score 2 or 3 on visit 2, 5 (31%) were infected on the 1st visit and remained infected. Not only did this culture protocol led to fewer samples to be cultured, but it may have had a predisposition to bacteria that produce a high inflammatory response, such as *S. aureus*. Unfortunately, *Staphylococcus aureus* is also known to be shed intermittently in milk samples, due to formation of micro-abscesses, potentially leading to no growth on a single milk sample (Sears et al., 1990). *S. aureus* is a contagious mastitis agent that is associated with chronic mastitis cases and has suboptimal cure rates by lactation intra-mammary infusion treatment (Sharma et al., 2011; Sol et al., 1997).

The low frequency of isolates can also be partly attributed to issues around sample storage. The samples were stored in a freezer from day 1 of sampling to the last day of the second round of farm visits. Repeated freeze-thaw cycles can reduce the survival of certain bacteria, particularly environmental mastitis pathogens, (Schukken et al., 1989). Unfortunately, there were some long power outages during the study period, likely leading to some repeated freeze/thaw cycles, despite our efforts to minimize this problem with large ice packs in the freezer.

For isolates recovered, results from this study agree with previous studies in Kenya which found *S. aureus* to be the predominant organism isolated (Gitau et al., 2014; Mureithi & Njuguna, 2016; Shitandi & Sternesjö, 2004). This result was contrary to findings from a different study in the same region (Mbindyo et al., 2020) that found *Streptococcus agalactiae* as the predominant agent, with *S. aureus* coming third after coagulase negative *Staphylococcus*.

The isolated organisms were resistant to penicillin and ampicillin, but had moderate susceptibility to a combination of ampicillin and cloxacillin (Ampiclox®) which are synthetic penicillins. Two out of 19 isolates were also resistant to cephalexin, a 1st generation cephalosporin. This finding indicated high resistance levels against beta-lactum antibacterials. The reason for this resistance could be due to prolonged use, since this class of antibacterials are among the oldest and have been in use for a long time, relative to most other classes of antibacterials. Most isolates were at least partially susceptible to gentamicin (an aminoglycosides) and tetracycline. Resistance for kanamycin on the first visit was low (3 out of 15 quarters), and proportions of susceptibility were fairly consistent between the two visits. Streptomycin susceptibility was high on the first visit (73%) but low on the second visit (17%). Based on first and second visit culture results, there was no substantial change in antimicrobial susceptibility. It was difficult to make inferential conclusions from the sensitivity results due to the small number of isolates from the samples. Due to several technical challenges in the laboratory, all antimicrobial resistance test results should be interpreted with caution.

5.5 Integration of Results from the Three Substantive Chapters

Stall hygiene and alleyway hygiene were identified as important factors associated with occurrence of subclinical mastitis in the baseline cross-sectional study (Chapter 2). These two variables were also among the recommendations given with the highest frequency among intervention farms in the trial described in Chapters 3 and 4, at 82% and 49%, respectively. Furthermore, these two variables had among the highest levels of compliance with their recommendations, at 83 and 64%, respectively. Therefore, these two variables contributed substantially to the farm improvements from the intervention, and the intervention was associated with improvement of quarter level mastitis on the second visit.

Bedding type and neck rail positioning were identified as important determinants of the discomfort index in the stalls (which is a function of stall hardness and hygiene). These variables were also identified as frequent areas of improvement for most SDFs on intervention farms. These findings would suggest that farmers' attention to bedding and neck rail positioning should lead to improvements in stall hygiene, and then improvements in stall hygiene (and alleyway hygiene) should lead to improvements in subclinical mastitis control.

5.6 Conclusions and Recommendations

From this study, it is evident that SDFs can reduce prevalence of subclinical mastitis by implementing integrated mastitis control and cow comfort recommendations. There remains a high prevalence of subclinical mastitis on SDFs, and this disease should continually be addressed as an important limitation to optimal productivity. Women and older persons (above 50 years of age) often play a key role in running of SDFs, and therefore their training should be enhanced further to realize better gains.

Since a validated scoring system for overall cow comfort does not exist, we used different components of cow comfort (stall hygiene and stall hardness) to build the discomfort index model. As such it was not possible to precisely assess the relationship between overall comfort of each cow and subclinical mastitis. There is need for a validated scoring system for cow comfort to be used as a standard in cow comfort studies such as this one that seeks to relate cow comfort to other factors impacting on the wellbeing of dairy cows.

Antimicrobial resistance is a reality on SDFs in developing countries such as Kenya. Resistance to drugs that are used for veterinary and human treatment, such as penicillins and cephalosporins, was noted. Farmers should be educated on antimicrobial stewardship to avoid them making the situation worse by indiscriminate and uninformed use of antibacterials in a bid to control mastitis. Finally, integrated mastitis control approaches should be incorporated in farmers' education programs to make them better aware and capable of controlling mastitis. This will reduce the need to use antibiotics and aid in the global campaign for antimicrobial stewardship.

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6. APPENDICES

6.1 Appendix 1: Recruitment script for research participants

Hello (name). My name is Edward Kariuki from the University of Prince Edward Island in Canada and University of Nairobi. I am calling to tell you about a research project looking at cow comfort on smallholder dairy farms in Kenya.

The Buuri/Naari Dairy gave me your name because you have at least 1 milking cow that calved in the last 2 months, don't have more than 4 milking cows, and you sell milk to Buuri/Naari Dairy. Can you confirm that you currently have at least 1 milking cow and sell milk to Buuri/Naari Dairy?

If yes to the eligibility criteria…continue

If no… I am sorry but you are not eligible for the project. Sorry to bother you. Thank you for your time.

The purpose of the project is to demonstrate the advantages of improving cow comfort and hygiene. If the project demonstrates that the benefits outweigh the costs, farmers should have the evidence that will convince them to implement changes in cow comfort and hygiene, leading to improved cattle health and productivity.

The project is being done in collaboration with Farmers Helping Farmers, and supervised by Dr. John

The research will take place on your farm.

The project involves us providing you with some free specific design and management advice for your cattle stalls. The management recommendations would be based on best management practices applicable to smallholder dairy farms in Kenya. We would visit your farm twice over the period of 2-3 months

Each visit will take approximately 1 -1 ½ hours for most farms.

Would you like to know more about the project? We could come to your farm in the next day or two.

If yes… set up a time and date to visit and confirm farm location and directions.
If no…thank the farmer for listening and ask their reason for not wanting to participate. If it is due to a misunderstanding of the study protocol or goals, give clarification and re-ask about coming to the farm.

6.2 Appendix 2. : Information letter for consent of research participants

We invite you to participate in a research project on housing management of dairy cattle.

Project Title: Cow comfort interventions on smallholder dairy farms in Kenya

A research team will be conducting the project, including:

Names:	**Affiliation**	**Contact Information**	**Role**
Dr. John VanLeeuwen	Professor at University of Prince Edward Island, Canada	jvanleeuwen@upei.ca	Canadian Project Leader
Dr. George Gitau	Professor at University of Nairobi, Kenya	gkgitau@uonbi.ac.ke	Kenyan Project Leader
Dr. Anne Muckle Dr. Shawn McKenna Dr. Greg Keefe Dr. Luke Heider	Professors at University of Prince Edward Island, Canada	cmuckle@upei.ca slmckenna@upei.ca gkeefe@upei.ca lcheider@upei.ca	Project Advisors
Dr. Edward Kariuki	Graduate Student at University of Prince Edward Island, Canada	karisedward@gmail.com	Project Implementer
Dr. Emily Kathambi	Hired veterinary consultant	kiuguek@gmail.com	Project Implementer
Ken Mellish	Farmers Helping Farmers	Ktm.mellish@pei.sympatico.ca	Project Advisor

Purpose of the project

The purpose of the project is to demonstrate the advantages of improving cow comfort and hygiene. If the project demonstrates that the benefits outweigh the costs, farmers should have the evidence that will convince them to implement changes in cow comfort and hygiene, leading to improved cattle health and productivity.

Voluntary Participation

Whether or not you take part is completely up to you. You may stop participating in the project at any time, without any consequences. You need only notify the Kenyan or Canadian project leaders. No reason for withdrawing from the project will need to be given to the people running the project. We will keep all information that we collect during this project confidential and anonymous, and destroy it after 5 years. We will ensure that you will not be identified in any of your responses to questions. We will identify you only by a number or a code name in the final records. Additional information on the project can be obtained from the research team.

Who can participate in the project?

You may participate in the project if you are an active member of the Buuri/Naari Dairy Farmers Cooperative Society in Meru County, you have a dairy cow that just calved in the last 2 months, and you have no more than 4 milking cows.

What does the project involve? What will you be asked to do?

The project involves us providing you with some free specific design and management advice for your cattle stalls. The management recommendations would be based on best management practices applicable to smallholder dairy farms in Kenya.

We want to assess the benefits of the enhancements, so we need a group of farmers who will be monitored, but not receive the enhancements initially. We would randomly select you to be in either the early or late enhancement group. We will return 2 months after the initial visit to do monitoring of your progress with the enhancement recommendations. Farms not receiving the recommendations on visit 1 will receive the recommendations on visit 2. You will get free pour-on dewormer for your cows on the first visit.

Possible Risks/Discomforts and Benefits:

The risks of harm from your involvement in this project are minimal. In fact, with making the recommended stall design and management changes, your cattle will likely be healthier and happier than if you were not involved in the project, with these benefits starting when the recommendations are implemented. These benefits will likely continue far into the future.

Conflict of Interest:

You and other smallholder dairy farmers in your community will benefit from the additional knowledge from this research, along with the animal health management advice and resources provided. None of the researchers will receive any direct benefits from the project. Funds for this project are from the Queen Elizabeth II Diamond Jubilee Scholarships, the Atlantic Veterinary College in Canada, the Nairobi Veterinary College, and Farmers Helping Farmers.

Compensation:

If you agree to participate in the project, you will also receive free advice on animal health management for your cattle to compensate you for your time and free pour-on dewormer for your cows.

There will be no cost to you for entering your animals in this study. You will not be charged for any of the procedures performed solely for the study's purposes. All unrelated costs for diagnosis, management and treatment of your animal are your responsibility. You will receive a report of the study findings through the Dairy Group to which you sell your milk.

Problems or Concerns:

The Research Ethics Board of UPEI has approved this research project. If you have any difficulties with, or wish to voice concern about, any aspect of this project, or the ethical conduct of this study, you may contact the UPEI Research Ethics Board for assistance at (902) 620-5104, or reb@upei.ca

6.3 Appendix 3: Consent form for research participants

Project Title: Prevention of bovine mastitis through a combination of cow comfort and mastitis interventions on smallholder dairy farms in Kenya

By signing this form, I agree that:

• I have read or have had the research information explained to me and understand the material in the information letter. □ Yes □ No

• I am at least 18 years of age and am a legal owner of animal(s) included in this research □ Yes □ No

• The possible harms and benefits of this project have been explained to me. □ Yes □ No

• I understand that my participation is voluntary. □ Yes □ No

• I understand that I have the freedom to withdraw at any time. □ Yes □ No

• I understand that I have the freedom not to answer any question. □ Yes □ No

• I am free now, and in the future, to ask any questions about the project. □ Yes □ No

• I understand my information will be kept confidential, within the limits of the law. □ Yes □ No

• I understand my name & address won't be released/printed without asking me first. □ Yes □ No

• I understand that I can keep a signed and dated copy of this consent form. □ Yes □ No

• I understand that I can contact the UPEI Research Ethics Board at (902) 620-5104, or by e-mail at reb@upei.ca if I have any concerns about the ethical conduct of this study. □ Yes □ No

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OVERALL PARTICIPANT CONSENT: □ Yes □ No

"I, (First and Last Name of the participant) ___________________________, am satisfied with the information provided to me about this project, and hereby consent to take part in this study."

PARTICIPANT AUTHORIZATION FOR FUTURE CONTACT: □ Yes □ No

"I also agree to be contacted again to take part in future visits of this project or future projects."

______________________________ ____________________
Signature Date

RESEARCHER:

Name of researcher who obtained consent: ___________________________

______________________________ ____________________
Signature Date

6.4 Appendix 4: Pre–intervention questionnaire for mastitis prevention project conducted in Naari, Meru County, Kenya, in 2020.

Farmer Number: ______________ Survey Visit Date: ______________ Interviewer Initials: ________.

A. Farm overview:

1. Gender of **principal farmer (person who takes care of the cows)**: male/female/both/other
2. Woman's education completed: ____ primary ___ secondary ____ college/university ___ n/a
3. Man's education completed: ____ primary ___ secondary ____ college/university ___ n/a
4. Woman's age: _____ years ___ n/a
5. Man's age: _____ years ___ n/a
6. Percent of total income coming from dairy production: ___< 50% ___50-75% ___> 75%

7a. Area of land owned: ________________ acres / hectares (circle units)

7b. Percent of land used for crop and fodder production for cattle?

8. Area of land rented/used (unpaid): ________________ acres / hectares (circle units)

9a. Have you attended any training on cow comfort in the last year ? Y/N

9b. Have you attended any training on mastitis control in the last year ? Y/N

B. Feeding - Normal feeding: Some feeds are only given seasonally. **Over the last month**, please check which of the following you fed to your cattle (**amounts not needed**).

Feed name	**Fresh cow (Q10)**	**Other milking cows (Q11)**
a. Napier grass		
b. Grass silage		

c. Maize silage		
c. Grass hay		
d. Desmodium		
e. Sweet potato vines		
f. Tree fodders –specify		
g. Other high protein forages – lucerne, leucana, – identify which one(s)		
h. Maize stover		
i. Banana leaves		
j. Other fodder – specify (eg. weeds) __________		
k. Dairy meal		
l. Wheat bran		
m. Maize germ		
n. Vitamin/mineral powder		
o. Vitamin/mineral block		
p. Other feeds –specify (eg. meal or cake) _____________		
q. Water available (always/sometimes)	A/S	A/S

12a. Do you usually feed **dairy meal or grain** to cows for the **month before calving**? __YES __NO

12b. If yes, do you increase amounts of dairy meal/grain during pre-calving month? __YES __NO

13a. Do you feed **vitamins/minerals** to cows during the **month before calving**? ___YES ___NO

13b. If yes, what brand?

Brand: _______________ (from bag: Ca:P ratio: ____ Selenium amount & unit: ______)

13c. If yes, how much is given to the cow? Amount (in tablespoons or grams per day): ___________

14. How much **dairy meal and/or grain** (eg. maize "jam") do you give cows on the **day they calve**?

a) dairy meal ____ kg in morning ____ kg in evening (digitize total)

b) other grain (specify)____________ ____ kg in morning ____ kg in evening (digitize total)

15a. In general, during the first **5 months after calving**, do you normally feed the same amount of **dairy meal or grain** per day to your cows? ___YES ___NO

b. If no, what factors affect how much dairy meal or grain you feed per day?____________________

16. At what height do you normally cut and feed your Napier grass for milking cows?

a. Cows (rainy season)		**b. Cows (dry season)**	
1. mostly < 1.0 meter	_______	1. mostly < 1.0 meter	_______
2. mostly < 1.5 meters	_______	2. mostly < 1.5 meters	_______
3. mostly < 2.0 meters	_______	3. mostly < 2.0 meters	_______
4. mostly > 2.0 meters	_______	4. mostly > 2.0 meters	_______

17a. For your **cows,** did you always have enough feeds over the last month? Yes_____ No_____

17b. If no, which feeds were inadequate (check all that apply)?

___Forages ___Grain or meals ___Vitamin-minerals ___Water ___Other(specify)_______

18. Were you giving cows less high-quality forage (legume, silage) than you wanted to? Yes __No _

19. During the last month, how many hours per day is the cow manger empty, on average? ______

20a. When did you last deworm your cows? _________________________________

20b. What did you use to deworm your cows? _________________________________

21a. Did the recently calved cow(s) have any health problem in the mo after calving? Yes __No __

21b. If yes, what was it? ____

22a. Did the recently calved cow(s) not want to eat her dairy meal or grain in the last mo? Yes __ No __

22b. If yes, what was the cause? ____

23a. Did the other milking cows not want to eat dairy meal or grain in the last year? Yes __ No __

23b. If yes, what was the cause? ____

24a. Did a vet service provider visit your farm for a sick animal in the past year? Yes __ No __

24b. If yes, for what disease(s) did the vet say? ____

C. Mastitis Prevention Management

25. a) When washing a cow's udder, is a different cloth used for each milking cow? Yes __No __

b) Is the udder dried before milking with a clean cloth or paper towel? Yes __No __

c) Is a different cloth or paper towel used for each cow when drying the udder? N/A___Yes __No __

d) Do you strip out milk on a black surface to look for mastitis before milking? Yes __No __

e) If you have > 1 milking cow, do you wash your hands between milking cows? Yes __No __

f) Do you use a teat dip after milking every milking? Yes __No __

g) Do you give fresh feed after milking every milking? Yes __No __

h) Do you use dry cow treatment when drying cows off prior to calving? Yes __No __

i) How many cows leaked milk in the last year? _____

j) How many times per day do you milk your cows? _____

k) At what times of the day do you milk your cows? _____ ______ ______

26. a) How many cases of mastitis did you have in the last year? _____

b) If you had mastitis, how many cases of mastitis did you treat in the last year? _____

c) If you treated for mastitis, how many cases of mastitis did not resolve after 1 treatment? __

d) If you had mastitis, is the mastitic teat milked before or after other teats? Before __ After___

e) If more than 1 cow, is the mastitic cow milked before or after other cows? Before __ After___

f) Have you ever had CMT used on your farm to look for mastitis? Yes____ No___

27. a) How many times did you have milk rejected in the last year? _____

b) If you had rejected milk, what were the reasons for rejection? _____________________________

D. Cow Stall Design and Management

28. How often do you **remove manure** from where the **milking cows** lie down?

a) more than once a day	b) once a day	c) every other day
d) twice a week	e) once a week	f) less than once a week

29. How often do you remove manure from the alleyway?

a) more than once a day b) once a day
c) every other day d) twice a week
e) once a week f) less than once a week

30a. Is bedding used where the milking cows lie down? Yes __No __ If no, go to 33.

30b. If yes, what kind of bedding is used where the milking cows lie down?

a) grass/hay b) straw c) sawdust d) crop waste e) loose soil/sand f) other (specify __________)

31. How often do you **remove wet bedding** from where the **milking cows** lie down?

a) more than once a day b) once a day
c) every other day d) twice a week
e) once a week f) less than once a week

32. How often do you **add new bedding** to where **milking** cows lie down?

a) more than once a day b) once a day
c) every other day d) twice a week
e) once a week f) less than once a week

For observation:

33. What is the type of floor where the milking cows lie down?

1) concrete 2) dirt 3) other (please specify: _________________)

34. Is the floor lumpy (have to lie on sticks, rocks, dirt chunks , etc.) Yes __ No __

35. Is water/urine/feces able to flow (by gravity) under udder where milking cows lie down? Y _N _

37. How is the drainage of the stall? (Slope of the floor surface, low spots for pooling of urine & water)

a. Poor b. fair c. good

38. Are there sharp objects in cow shed that may injure the cattle (eg nails)? Yes __ No __

39. Is the roof appropriate (observe – no holes, extends to cover udder area)? Yes __No _

40. Condition of alleyway (ability to walk without stepping on manure)

a) muddy b) fair c) clean

E. Stall dimensions and conditions: For Measurement and Assessment

	a. Total Width Good (Y/N/M)	b. Total Length Good (Y/N/M)	c. Body Length Good (Y/N/M)	d. Brisket Board Good (Y/N/M)	e. Neck Rail Good (Y/N/M)	f. Forw/side Lunge Good (Y/N/M)	g. Leg Space Good (Y/N/M)	h. Knee Impact Good (Y/N/M)	i. Knee Wetness Good (Y/N/M)
41									
42									
43									
44									

For measurements a,b,c,e, see weight of the animal, compared to the chart in the handbook.

For example, for Total width,

if the width is within 10% of what it is supposed to be, the answer is Y;

if the width is 20% narrower or wider than what it is supposed to be, the answer is N;

if the width is between 10-20% of what it is supposed to be, the answer is M;

Putting numbers on this (and rounding to nearest ½ cm), for a 400 kg cow, the total width is supposed to be 96.5 cm (halfway between 91 and 102 cm).

> 10% of 96.5 is 9.65, or round to 9.5. So within 10% is 96.5-9.5 to 96.5+9.5 or 87 to 106
>
> 20% of 96.5 is 19.3, or round to 19.5. So within 20% is 96.5-19.5 to 96.5+19.5 or 77 to 116
>
> if the width is within 10% (87 to 106), the answer is Y;
>
> if the width is 20% narrower or wider than what it is supposed to be (77 to 116), the answer is N;
>
> if the width is between 10-20% of what it is supposed to be, (77-87 or 106-116), the answer is M.
>
> If you want to round to the nearest whole cm, that is fine too, and easier math.

Total length should be from the back of the stall (curb or posts) to the front of the stall (usually boards)

Body length should be from the back of the stall (curb or posts) to the brisket board, if one, or stall front

Neck rail is good if present and correct height (see chart) and rounded

For measurements d,f,g,h,i weight of the animal not relevant, so subjective assessment.

Brisket board is good if present and correct height (5-15 cm high) and rounded (not sharp edge)

Lunge space is good if cow head can easily (Y), marginally (M) or not (N) fit in front/side lunge space

Leg space is good if cow legs can easily (Y), marginally (M) or not (N) fit in leg space (20-25 cm)

Knee impact is good if you can comfortably (Y), marginally (M) or not (N) fall to your knee in udder area (don't bother if obviously hard – don't need to hurt your knees)

Knee wetness is good if your knees are dry (Y), slightly wet (M) or wet (N) when staying on your knees in udder area for 20 seconds (don't bother if obviously wet – don't need to soil your knees unnecessarily)

G. Milking Cow Health and Productivity Checks (only cows not to be sold/dried off in next 2 months)

Examination of Cows:	Cow1 (Q46) ID________	Cow2 (Q47) ID________	Cow3 (Q48) ID_______	Cow4 (Q49) ID_______
a. "Approximate age (years)"				
b. "Number of calvings"				
c. "Last calving date"				
d." Current daily milk yield (kg/d)"				
e. "Yesterday was typical day for milk?"	Y/N	Y/N	Y/N	Y/N
f. "Peak milk production?" (kg/d)				
g. "Leak milk before?" If yes, which quarter?	Y/N ______	Y/N ______	Y/N ______	Y/N ______
h. "Amount dairy meal (or grain) fed?" (kg/d)				
i. "Is she thinner now than she was a mo ago?"	Y/N	Y/N	Y/N	Y/N
j. "Not eat for at least a day in last month?"	Y/N	Y/N	Y/N	Y/N
k. "Mastitis in last month?"	Y/N	Y/N	Y/N	Y/N
l. "Mastitis in last 12 months?"	Y/N	Y/N	Y/N	Y/N
m. "Hear her hit wood when standing up?"	Y/N	Y/N	Y/N	Y/N
n. "Lie down in places other than stall?"	Y/N	Y/N	Y/N	Y/N
o. "Odd stall use (perch, stand idle, backwards, etc.)?"	Y/N	Y/N	Y/N	Y/N
p. "Vaccinated for BVDV in last couple years?"	Y/N	Y/N	Y/N	Y/N

q. Neck lesion (0, 1, or 2: chart)	0 / 1 / 2	0 / 1 / 2	0 / 1 / 2	0 / 1 / 2
r. Hock lesion (0, 1, 2 or 3: chart)	0 / 1 / 2 / 3	0 / 1 / 2 / 3	0 / 1 / 2 / 3	0 / 1 / 2 / 3
s. Carpus lesion (0, 1, 2 or 3: chart)	0 / 1 / 2 / 3	0 / 1 / 2 / 3	0 / 1 / 2 / 3	0 / 1 / 2 / 3
t. Udder hygiene score (1-5)				
u. Leg hygiene score (1-5)				
v. Stall hygiene score (1-5)				
w. Lameness (see walk: absent, mild or severe)	A / M / S	A / M / S	A / M / S	A / M / S
x. Breed				
y. Weight (kg)				
z. Height (cm)				
az. Body condition score (1-5; ½ point scale)				
ab. TPR/physical exam Normal / Abnormal? (manure, feet, skin, lymph nodes, eyes, rumen)	N / A	N / A	N / A	N / A
ac. CMT (circle CMT result if milk looks abnormal as well)	LF LH RF RH — — — —	LF LH RF RH — — — —	LF LH RF RH — — — —	LF LH RF RH — — — —

50. Milk taken? Y/N Y/N Y/N Y/N

6.5 Appendix 5. Post –intervention questionnaire for mastitis prevention project conducted in Naari, Meru county, Kenya in 2020.

Farmer Name: Farm Number: Phone #:

Survey Visit date: Interviewer Initials:

	Cow #1.1 ID_______	Cow #1.2 ID________	Cow #1.3 ID_______	Cow #1.4 ID_______
a. Body condition score				
b. TPR/physical exam Normal /Abnormal? Pathological? (manure, feet, skin, lymph nodes, eyes, rumen)	N / A	N / A	N / A	N / A
c. Udder hygiene score (1-5)				
d. Leg hygiene score (1-5)				
e. Stall hygiene score (1-5)				
f. Lameness (absent, mild or severe)				
g. Neck injuries score (1-3)				
h. Carpal injuries score (1-3)				
i. Hock injuries score (1-3)				
j. Mastitis (absent, mild or severe)				
k. Subclinical mastitis (CMT)	*LF__RF__* *LB__RB__*	*LF__RF__* *LB__RB__*	*LF__RF__* *LB__RB__*	*LF__RF__* *LB__RB__*
l. Milk production yesterday (kg/day)				
m. Yesterday was typical day for milk?				

2a. How many of the given recommendations did you complete?

a. none b. some c. most d. all

2b. If none, why not?...

3. How well do you feel you completed the given recommendations?

a. fair b. good c. very good d. excellent

4a. Were there any recommendations that were harder to complete than others?

Yes…… No……

4b. If yes, which ones?...........................

5. When did you start making the recommended changes made? Within…

a. 1 day b. 1 week c. 1 month

6. How long did it take to make all the changes to the milking cow stalls?

a. a few hours b. 1 day c. a few days d. 1 week 3. More than a week

7. What are the challenges you encountered in making the changes?

____

8a. Were there financial costs to make these changes? Yes…… No……

8b. If yes, how much?...

9a. Do you think you are well versed with the cow comfort requirements now? Yes… No…

9b. If yes, have you advised anyone else? Yes……. No……….

9c. If no, why not?..

Detailed assessment:

Type of Change Recommended	a. Recommendation made 0. None 1. Minor 2. Major	b. Recommendation compliance 0. Not done 1. Done partly 2. Done well	c. Score (equals a. times b.)
10.Roof water			
11.Surface Water			

12.Floor
soft/dry

13.Floor flat

14.Total width

15.Total length

16. Leg space

17.Lunge space

18.Neck rail

19.Brisket board

20.Alley clean

20.Sharps fix

21. Mastrite use

22. Milking
palour clean/dry

23.Wash
hands- soap &
water before
milking

24. Different
towel@ cow